Untargeted metabolomics reveal sex-specific and non-specific redox-modulating metabolites in kidneys following binge drinking

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Graphical abstract

Abstract

Binge drinking is a growing health concern among all age groups. The ability of individuals to handle ethanol in their systems differs not due to differences in enzyme expression but also due to other factors. Vital organs including brain, liver, heart, and kidneys are at high risk following repeated exposure to high concentrations of ethanol from binge drinking. The impact of chronic binge drinking on kidneys is not well studied. Using a mouse model of chronic binge drinking, we have identified major metabolic alterations that could set the stage for detrimental effects in the kidneys of male and female mice. We have deciphered that even though there are pathway overlaps, the different sexes exhibited unique and divergent metabolic pathway dysregulations as per the metabolite panels following binge drinking. We have reported that binge drinking could negatively influence renal redox homeostasis in both sexes through the regulation of different metabolite clusters. In male mice by downregulation of pantothenic acid and riboflavin synthesis,

Keywords
- chronic binge drinking
- ethanol metabolism
- metabolites
- untargeted metabolomics
- renal complications

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Introduction

Binge drinking is a major health and safety issue not only in the US but worldwide. According to the National Institute of Alcohol Abuse and Alcoholism, over 20% of the US population including adults and teens engage in binge drinking. Based on NIAAA’s definition, binge drinking is a type of drinking that leads to an elevation of blood alcohol concentration to reach 0.08% in a relatively short time span (2 h). Binge drinking results in hepatic, cardiorenal, and CNS complications (Altura & Altura 1984, Molina 2014, Gilbert & Zemore 2016, Parker et al. 2018, Curtis et al. 2019, Yussof et al. 2020). Organ damage due to binge drinking begins with the metabolism of excess alcohol as it enters hepatic cells from portal circulation after being absorbed from the gut (Zakhari 2006, Bradford 2007, Teplova et al. 2017, Kubiak-Tomaszewska et al. 2020, Yue et al. 2021). The first two steps in ethanol metabolism in hepatic tissue involve (i) the conversion of ethanol to acetaldehyde in the presence of alcohol dehydrogenase and (ii) then to acetate in the presence of aldehyde dehydrogenase (Zakhari 2006, Bradford 2007, Teplova et al. 2017, Kubiak-Tomaszewska et al. 2020, Yue et al. 2021). In both these steps, nicotinamide adenine dinucleotide (NAD+) acts as a co-factor and undergoes reduction to form NADH (Zakhari 2006, Bradford 2007, Teplova et al. 2017, Kubiak-Tomaszewska et al. 2020, Yue et al. 2021). With binge drinking, the NAD+ homeostasis is altered but what happens to the metabolite landscape or metabolome in specific tissues is not well known.


What has not been given enough focus is the early metabolic changes which could be a detrimental factor leading to target organ damage irrespective of the subject’s health status or underlying disease condition. Following binge drinking, ethanol undergoes major metabolism in hepatic tissue to get detoxified into its acetate metabolite which then can be transported in the circulation and could get converted in target tissues to Acetyl CoA (Zakhari 2006, Bradford 2007, Kubiak-Tomaszewska et al. 2020). Under aerobic conditions, ethanol has three major pathways to undergo metabolism (i) bioconversion to acetaldehyde and acetate with the help of enzymes – ADH and ALDH2, respectively, (ii) CYP2E1-mediated bioconversion, and (iii) Catalase-mediated metabolism. Among these three routes, ADH/ALDH2-mediated metabolism is the preferred route (Zakhari 2006, Bradford 2007, Kubiak-Tomaszewska et al. 2020). Compared to all other organs, the liver has abundant expression of alcohol metabolizing enzymes, and following binge, the hepatic system is heavily burdened (Fogel et al. 1991, Traves et al. 1995, Robin et al. 2005, Zakhari 2006, Bradford 2007, Comporti et al. 2010, Mallikarjuna et al. 2010, Okuda et al. 2018, Kubiak-Tomaszewska et al. 2020). This could result in the escape of metabolized and incompletely metabolized ethanol reaching the circulation and finally the kidneys as a part of the excretion process, which is an adaptive process to safeguard our system from excess ethanol toxicity (Hirsch et al. 1994, Cecchin & De Marchi 1996, Jung et al. 2012). Along with the normal excreting role, the kidneys play an equally important role in detoxifying chemical agents like ethanol in coordination with the liver (Hirsch et al. 1994, Cecchin & De Marchi 1996, Jung et al. 2012, Parker et al. 2018). Binge alcohol drinking could not only cause injury to the renal system per se but also influence cardiovascular and other vital systems (Cecchin & De Marchi 1996, Bradford 2007, Jung et al. 2012, Molina 2014, Zhong et al. 2014, Parker et al. 2018). Therefore, it is crucial to understand the shift in the renal metabolome...
following binge drinking. Using a well-established binge drinking model (Thiele & Navarro 2014, Chen et al. 2015, Schweitzer et al. 2016, Truitt et al. 2016) Thiele, 2014 #102), we have identified the change in the renal metabolome in both sexes. Interestingly, our study demonstrates the change in metabolites in renal tissue occurs in a sex-dependent manner. It is very crucial to understand the regulation of metabolites in different sexes following binge drinking as this could provide insights into the treatment regimen and identify alcohol-induced early renal pathological mechanisms.

## Materials and methods

### Animal use

C57BL/6j mice were purchased at the age of 8 weeks from The Jackson Laboratory. Male and female mice (n=3 per sex per treatment, total 12 mice) were used for this study. Following receipt into the University of Illinois vivarium, mice were housed in a reversed light/darkness cycle room for 2 weeks to acclimate to the change in the light cycle. At the age of 10 weeks, mice underwent the drinking in the dark procedure for 6 weeks as described later. Mice were 16 weeks old at the time of tissue collection. We followed the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. All the procedures were approved by the University of Illinois at Chicago Animal Care Committee.

### Chronic binge drinking model

Mice were individually housed in a temperature- and humidity-controlled room with a 12 h reversed light/darkness cycle (lights off at 10:00 h), with food and water available ad libitum, except a water bottle was not available during the limited-access ethanol drinking. The design for chronic binge drinking was based on a well-established method (Chen et al. 2015, Satta et al. 2018). In brief, 20% ethanol in tap water (or water, as a control) was provided as a single bottle in the home cage from 13:00 to 17:00 h on Monday to Thursday of each week. Ethanol was not provided from Friday to Sunday. Each drinking cycle was repeated weekly for 5 additional weeks. The amount of ethanol consumed was calculated as grams (g) ethanol per kg body weight. Mice were euthanized immediately after the ethanol drinking session on Thursday of the 6th week and kidneys isolated and frozen. Processed tissue samples were shipped on dry ice to Creative Proteomics (Shirley, NY, USA) for untargeted metabolomics.

### UPLC-TOF-MS technology

For untargeted metabolomics, Creative Proteomics performed ultra performance liquid chromatography (UPLC) with time-of-flight mass spectrometry (ESI-TOF-MS). Following sample separation, mass spectrometry was performed using positive and negative ion modes using a previously well-established procedure at the facility (Vinayavekhin & Saghatelian 2010, Fiehn 2016, Schrimpe-Rutledge et al. 2016).

### Sample analysis and statistical methods employed

Once the raw data were obtained, the individual metabolites were identified using Compound Discover and Thermo 3.0 system depending upon their mass-to-charge ratios (m/z) and also based on the ion signal retention times. A SIMCA-P program (version 14.1)-based multivariate analysis was performed after combining the emerging ions from both positive ion (ESI+) and negative ion (ESI−) modes. Both unsupervised and supervised methods with the help of principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA)/orthogonal partial least squares discriminant analysis (OPLS-DA), respectively, were employed for identifying the target metabolites and for data visualizations. Metabolites with variable importance in projection (VIP) values >1.5 and a test-based determination of significance with a P value < 0.05 were selected as significantly altered targets. These determinations were done at Creative Proteomics facility and were based upon previously established methods (Vinayavekhin & Saghatelian 2010, Fiehn 2016, Schrimpe-Rutledge et al. 2016).

## Results

### Ethanol consumption in the chronic binge drinking model

The long-term binge drinking is based upon a repeated pattern of drinking for 6 weeks from Monday to Thursday as previously described (Chen et al. 2015, Satta et al. 2018). The 20% ethanol drinking group did not have access to ethanol from Friday to Sunday each week and this was continued for up to 6 weeks. The control and 20% ethanol group were euthanized on Thursday after 17:00 h in the 6th week immediately following ethanol consumption (Fig. 1A). Male mice drank an average of 5.4 g/kg per 4 h session in the first week and ~7–8 g/kg ethanol per 4 h
session in subsequent weeks. As shown previously, female mice consumed more ethanol than males, with an average of 8 g/kg ethanol per 4 h session in the first week and ~10–11 g/kg per 4 h session in subsequent weeks. Total ethanol consumed per week ranged from ~22–32 g/kg for males and 33–43 g/kg for females (Fig. 1B). There was a significant sex difference in weekly ethanol consumption, with females consuming more than males (two-way RM ANOVA: effect of sex, $F(1, 4) = 13.06, P = 0.02$; effect of week, $F(5, 20) = 7.415, P = 0.0004$). Total ethanol consumed per kg body weight at the end of the 6 weeks was also higher in females than in males (Fig. 1C). There was a significant sex difference in weekly ethanol consumption, with females consuming more than males (two-way RM ANOVA: effect of sex, $F(1, 4) = 13.06, P = 0.02$; effect of week, $F(5, 20) = 7.415, P = 0.0004$). Total ethanol consumed per kg body weight at the end of the 6 weeks was also higher in females than in males (Fig. 1C, females, 234.5 ± 23.7 g/kg; males, 170.4 ± 19.6 g/kg; Student’s $t$-test: $t(4) = 3.619, P = 0.02$). When corrected for body weight, females also drank on average more water than males (females: 60.4 ± 4.2; males: 43.6 ± 6.9 g/kg/4 h; $t(4) = 3.62, P = 0.02$).

Metabolomics-based analysis demonstrated differences in metabolite levels in both males and females following chronic binge drinking

Metabolites were obtained from both male and female kidneys in negative and positive ion modes (ESI− and ESI+). The data analysis using PCA, PLS-DA, and OPLS-DA revealed no overlap in the metabolite pattern in control (water) and chronic binge ethanol drinking groups (Supplementary Fig. 1, 2, 3, and 4, see section on supplementary materials given at the end of this article). Females that drank ethanol demonstrated a clear difference from the female control group based on all three analyses in comparison to males, indicating that the change in metabolite levels in response to ethanol occurs in a much more robust manner in females (Supplementary Fig. 3 and 4) than in males (Supplementary Fig. 1 and 2).

The metabolite sets exhibited a distinctive pattern in males and females following chronic binge drinking

The metabolite sets or clusters with substantial enrichment ratio and with statistical significance of $P < 0.05$ values were different in both males (Figs. 2 and 3) and females (Figs. 4 and 5). As per the service provider (Creative Proteomics), the enrichment analysis was performed based on the mapping of well-annotated metabolite sets available on Human Metabolome Database (HMDB). The enrichment ratio is based on observed hits to expected hits and the size of the cluster is based upon fold change in enrichment with significance represented by $P$-value. The transition of color between yellow to red indicates low to high significance, respectively. We have selected clusters based on the high enrichment ratio (size of the cluster) and significance ($P$-value) indicated by red or intense red color. In males, among the major clusters that were altered, the top three sets with significant enrichment ratio were (i) pantothenate and CoA...
biosynthesis, (ii) steroid hormone biosynthesis, and (iii) pyrimidine metabolism in positive ion mode. For negative ion mode, the top three significantly altered clusters include (i) purine metabolism, (ii) pyrimidine metabolism, and (iii) primary bile acid biosynthesis. The metabolite clusters demonstrated quite distinct pattern in females. For positive ion mode in females, the top three regulated metabolite sets were (i) glycine, serine, and threonine metabolism (amino acids); (ii) valine, leucine, and isoleucine biosynthesis; and (iii) aminoacyl t-RNA biosynthesis. In negative ion mode, the top three regulated metabolite clusters were (i) lysine degradation, (ii) tryptophan metabolism, and (iii) steroid hormone biosynthesis. From these observations, steroid hormone biosynthesis is found to be a common metabolite cluster significantly altered in both males and females.

The significantly altered independent metabolites confirm the cluster analysis data in both male and female binge drinking groups

The heatmap-based cluster analysis demonstrated a significant decrease in pantothenic acid, pregnenolone, and uracil among the major metabolites belonging to top clusters in pantothenate and CoA biosynthesis, steroid hormone biosynthesis, and pyrimidine metabolism in positive ion mode in males (Fig. 6). In the negative ion mode, glutamic acid/glutamate which is the major nitrogen donor for purine and pyrimidine nucleotides, uridine – a pyrimidine ribonucleoside, and deoxycholylphenylalanine were the major metabolites downregulated (Fig. 7). These metabolites belonged to purine metabolism, pyrimidine metabolism, and primary bile acid biosynthesis clusters, respectively. Also, the metabolites uric acid and ammonia aspartate (conjugate) which are metabolic breakdown products of purine and pyrimidine metabolism were upregulated in negative ion mode in males following binge drinking, confirming the cluster analysis data. In females (Fig. 8), for positive ion mode, threonine levels were altered. Threonine forms the crucial link and major intermediate between glycine,
serine, and threonine breakdown and valine, leucine, and isoleucine biosynthesis (Dong et al. 2018, Sahoo et al. 2021, Yu et al. 2021). We have observed a decrease in threonine expression. The cluster aminoacyl t-RNA biosynthesis depends upon the availability of amino acids (Rubio Gomez & Ibba 2020). With the reduction in threonine, there could be the downregulation of t-RNA biosynthesis of aminoacyl derivatives of threonine per se and threonine-dependent amino acids; valine, leucine, and isoleucine. This correlates well with the reduction in t-RNA biosynthesis cluster which we observed. For negative ion mode in females with binge drinking (Fig. 9), we observed an increase in metabolite levels of amino adipic acid, the major intermediate in lysine degradation, and its precursor adipic acid. Tryptophan metabolism was another major metabolite cluster regulated in females. Tryptophan can be broken down either via the 5-hydroxytryptophan pathway to serotonin or it could get converted through N-formylkynurenine and finally form kynurenine (Beal et al. 1990, Fukuda 2014, Comai et al. 2016, Matsuoka et al. 2017, Rabbani & Barik 2017, Ormstad et al. 2018). Among the highly regulated metabolites, formylkynurenine was upregulated, whereas hydroxytryptophan was downregulated in females with binge drinking (Fig. 9). Regarding the steroid hormone biosynthesis cluster, dehydroepiandrosterone sulfate and 11-hydroxytestosterone, the precursor and ligand for androgen receptor were increased in the binge drinking group. Our data from both cluster analysis and individual metabolite panels demonstrate strong coordination and validate each other.

Discussion

Excessive alcohol use poses a serious health threat. With binge drinking, injury is incurred to multiple organ systems in a short time span. To date, the focus has been on hepatic injury and addiction-related CNS disorders (Altura & Altura 1984, Molina 2014, Parker et al. 2018), but there has been less attention on the drastic metabolic changes underlying binge drinking on target organs like the kidneys nor are there any published reports on the impact of binge drinking on the renal metabolome. Based
on the untargeted metabolomic analysis presented here, we have identified a major shift in metabolome following binge drinking in a sex-dependent manner. Interestingly, among the top three significantly altered metabolic pathways in males, in both the positive and negative ion modes, pyrimidine metabolism is the most commonly regulated pathway. In-depth evaluation of independent metabolites revealed that uracil, a nucleic acid base, and its ribonucleoside, uridine, was downregulated in positive and negative ion modes, respectively, highlighting the impact of binge drinking in cellular RNA synthesis. Independently, in both positive and negative ion modes, metabolites involved in pantothenate and CoA biosynthesis, and steroid hormone biosynthesis for the positive ion mode and purine metabolism and bile acid biosynthesis for the negative ion mode were affected in males (Figs. 6 and 7). The independent metabolites belonging to these metabolic clusters include pantothenic acid (+) (vitamin B5), also a precursor for CoA synthesis, pregnenolone (+) the precursor for steroid hormones, glutamic acid/glutamate (−) excitatory neurotransmitter, and deoxycholylphenylalanine (−), a bile acid derivative, all these metabolites were all downregulated (Fig. 6 and 7). CoA is a major co-factor involved in the bioconversion of substrates for the TCA cycle and mandatory for fatty acid metabolism (Zhang et al. 2007, Qian et al. 2015, Snyder et al. 2015, Satoh et al. 2020). Pantothenic acid (vitamin B5), which is a known precursor for acetyl CoA formation, was decreased with binge drinking in males, which could lead to a potential decrease in the provision of TCA cycle substrates, specifically via fatty acid metabolism (Zhang et al. 2007, Qian et al. 2015, Snyder et al. 2015, Satoh et al. 2020). Pregnenolone is a downstream metabolite of cholesterol and precursor for the synthesis of the majority of steroid hormones including progesterone, corticosterone, and aldosterone (Aguilera et al. 1996,}

Figure 7
Altered metabolites in negative ion mode for kidneys of male binge drinking group. The metabolites (in negative ion mode) significantly altered in female kidneys following binge drinking were expressed using heatmap. The ones upregulated are expressed in red and the ones downregulated are expressed in green with volcano plots as inserts.
The male binge-drinking group demonstrated a decrease in pregnenolone. A downregulation of pregnenolone could potentially lead to carbohydrate, sodium, and water imbalance in binge-drinking male mice, an effect due to downregulation of glucocorticoid and mineralocorticoid in the body (Fig. 6).


The formed glutamate can also undergo bioconversion to α-ketoglutarate, a major substrate for the TCA cycle (Shibayama et al. 2007, D’Alessandro et al. 2011, Reynolds et al. 2014, Cetinbas et al. 2015, Fu et al. 2017, Watanabe et al. 2021). A downregulation of glutamine/glutamate pathway could lead to suppression of (i) nucleotide (both purine and pyrimidine) biosynthesis, (ii) excitatory neurotransmission (due to reduced glutamate), and (iii) TCA cycle substrate availability (due to reduced formation of α-ketoglutarate from a glutamate) in male mice (Fig. 7).

The bile acid derivative, deoxycholylphenylalanine is also downregulated in binge-drinking male mice indicating reduced bile acid formation and interfering with lipid digestion and cholesterol breakdown (Fig. 7).

In females, the top three enriched metabolite sets or clusters were the amino acid triads – glycine, serine, and threonine metabolism; valine, leucine, and isoleucine biosynthesis; and aminoacyl t-RNA biosynthesis in the
positive ion mode and lysine degradation, tryptophan metabolism, and steroid hormone biosynthesis for the negative ion mode (Fig. 4). When we analyzed the individual component/components belonging to these major clusters, we identified threonine as a major connecting link between glycine, serine, and threonine metabolism; valine, leucine, and isoleucine biosynthesis; and aminoacyl t-RNA biosynthesis clusters (Dong et al. 2018, Rubio Gomez & Ibba 2020, Sahoo et al. 2021, Yu et al. 2021) (Fig. 4). In glycine, serine, and threonine metabolism, all these amino acids can undergo bioconversion to each other, and threonine can serve as the final breakdown products of the other two (Dong et al. 2018, Cheng et al. 2019, Sahoo et al. 2021) (Fig. 4). The amino acids glycine and serine via threonine can form the precursor for biosynthesis of valine, leucine, and isoleucine (Dong et al. 2018, Cheng et al. 2019, Sahoo et al. 2021). The formation of threonine from glycine and serine is considered the first step in this biosynthetic pathway, which highlights the significance of threonine in both the breakdown and biosynthesis of other amino acids (Dong et al. 2018). Amino acids coordinate with t-RNA to form the aminoacyl t-RNA formation during the process of protein biosynthesis (Rubio Gomez & Ibba 2020). Reduction in major amino acid intermediate threonine could result in a debilitated protein synthesis due to less availability of valine, leucine, and isoleucine along with threonine itself (Cheng et al. 2019, Sahoo et al. 2021). Here with the aid of untargeted metabolomics, we found a decrease in biochemical pathways leading to protein biosynthesis in females following binge drinking. In negative ion mode, lysine degradation, tryptophan metabolism, and steroid hormone biosynthesis were the major pathways regulated in female mice following binge drinking (Fig. 5). We observed an increase in aminoadipic acid and its further breakdown product, adipic acid. Extensive lysine breakdown could lead to an increased formation of aminoadipic acid, which is also a marker for oxidative stress (Fig. 9). Regarding tryptophan metabolism, hydroxytryptophan demonstrated a
Hydroxytryptophan is a major intermediate in the formation of serotonin, which is a major chemical neurotransmitter with prominent physiological function (Maffei 2020). In the presence of hydroxytryptophan decrease, another fate of tryptophan is to channelize into kynurenine (Giil et al. 2017, Maffei 2020). We observed an increase in formylkynurenine levels, a kynurenine derivative (Fig. 9). Enhanced tissue-specific kynurenine levels are known to cause cognition deficits in CNS (Fukuda 2014, Beal et al. 1990, Suzuki & Mori 1992) and are elevated during Alzheimer's (Giil et al. 2017) and also in cardiovascular disease like coronary artery disease (Zapolski et al. 2020, Sudar-Milovanovic et al. 2022, Wang et al. 2022). The impact of enhanced kynurenine levels in kidneys is yet to be studied. The third metabolite cluster that was altered in the female binge drinking group was that of steroid hormone biosynthesis (Fig. 5). The two major individual metabolites that demonstrated enhanced levels were dehydroepiandrosterone sulfate and 11-hydroxytestosterone (Fig. 9). Dehydroepiandrosterone sulfate and 11-hydroxytestosterone are the precursors for male sex hormone biosynthesis and ligand for testosterone receptor, respectively (Turcu et al. 2014). An enhancement in sex hormone synthesis following binge drinking in females could bring about systemic endocrine abnormalities along with changes in metabolism. Apart from the metabolites which are components of the top regulated clusters we also identified independent metabolites which could influence metabolic health. In males, along with the decrease in pantothenic acid, we also observed a decrease in riboflavin in the negative ion mode. Like pantothenic acid's role in cellular antioxidant defense, riboflavin also contributes positively toward the cellular antioxidant mechanism directly and indirectly (Appenroth et al. 1996, Alam et al. 2015). Riboflavin (Vitamin B2) acts as the major co-factor in glutathione reductase enzyme activity. Glutathione could effectively perform its antioxidant capacity in its reduced form and lack or deprivation of riboflavin could lead to a reduced glutathione status disrupting cellular antioxidant capacity. A decrease in riboflavin levels has also been associated with lipid peroxidation which is a major contributing factor for enhancing oxidative stress leading to cardiorenal complications (Ashoori, 2014 #110). We also observed an increase in uric acid levels in binge-drinking male mice in negative ion mode. Uric acid by itself is a major factor which could promote reactive oxygen species generation and inflammation in the tissues (Battelli et al. 2016, Martorell et al. 2021, Si et al. 2021). In the female binge-drinking group in positive ion mode, along with adipic acid, we observed an increase in xanthosine levels (Fig. 8). Xanthosine is a precursor for uric acid biosynthesis, which is a contributing factor for oxidative stress (Battelli et al. 2016, Martorell et al. 2021). Multiple studies have demonstrated and validated the role of uric acid, as a toxin and independent risk factor for cardiorenal disease and exacerbating existing cardiorenal complications by upregulating pro-inflammatory pathways, inducing oxidative stress and promoting various forms of cell death distorting cell to cell cross-talk and metabolic homeostasis (Kanbay et al. 2011, Riegersperger et al. 2011, Stellato et al. 2012, Chaudhary et al. 2013, Kaufman and Guglin 2013, Akhigbe et al. 2022, Gherghina et al. 2022) (Yang, 2019 #113). Another independent oxidative stress marker which demonstrated significant upregulation in the female binge drinking group in positive ion mode was N-nitrosoglutathione (Fig. 8). Augmented levels of N-nitrosoglutathione are an indication of enhanced nitrosative stress occurring during binge drinking in females. N-nitrosoglutathione could itself act as a signaling molecule altering redox potential (Rosales, 2014 #121). Glutathione by capturing the excess reactive nitric oxide species from respective proteins which are nitrated tries to maintain the nitrosative stress homeostasis (Yoon, 2021 #117; Rosales, 2014 #121; Larrick, 2019 #119; Kronenfeld, 2015 #120; Chatterji, 2021 #118). As a result, it could deplete available glutathione levels which could be detrimental under conditions of binge drinking. This data emphasize the risk for female binge drinkers in comparison to male binge drinkers.

Adipic acid came up in top upregulated metabolite in both positive and negative ion modes in binge females. Along the existing diverse features, we observed in binge-drinking male and female mice, one of the unique and converging features is that binge drinking causes changes in metabolites that could alter or distort metabolic and redox homeostasis in both sexes. Binge drinking brings about this outcome in males and females by altering different metabolites or clusters.

Earlier studies have demonstrated the mitochondrial protective function of pantothenic acid (Tahiliani & Beinlich 1991, Williams et al. 2013, Sm et al. 2018, Subramanian et al. 2021, Subramanian et al. 2023). Pantothenic acid is a major precursor for CoA synthesis (Tahiliani & Beinlich 1991, Williams et al. 2013, Sm et al. 2018, Subramanian et al. 2021, Subramanian et al. 2023). Pantothenic acid-mediated CoA biosynthesis is required for the effective metabolism of all substrates by converting them to Acetyl CoA which is the preferred form for
mitochondrial utilization (Tahiliani & Beinlich 1991, Williams et al. 2013, Sm et al. 2018, Subramanian et al. 2021, Subramanian et al. 2023). Along with CoA synthesis, pantothenic acid has also been demonstrated to regulate glutathione biosynthesis (Slyshenkov et al. 2004). In Jurkat cells, pantothenic acid supplementation is known to augment endogenous glutathione levels and protect the cells against peroxide-induced oxidative damage (Slyshenkov et al. 2004). Additionally, correlation studies in the brain have demonstrated that deficiency in pantothenic acid could lead to a decrease in CoA-dependent mitochondrial enzymes which are major regulators of the citric acid cycle like isocitrate dehydrogenase and succinyl-CoA synthetase (Xu et al. 2020). These findings uphold the significance of pantothenic regulation in the mammalian system in not only regulating CoA but also glutathione and mitochondrial enzyme activity regulation maintaining redox homeostasis. Additionally, CoA per se has been demonstrated to be upregulated in response to pathogenic infection and metabolic stress (Gout, 2018 #112; Tsuchiya, 2018 #111). CoA can act as the precursor for the biosynthesis of thioesters which possess antioxidant properties (Gout, 2018 #112; Tsuchiya, 2018 #111). Thus, reducing pantothenic acid or CoA or both ethanol through binge drinking is suppressing the overall antioxidant capacity of the cells.

Ethanol excess, by downregulating pantothenic acid, could potentially deter mitochondrial health and redox homeostasis in multiple ways. We are the first to report this connection between ethanol consumption and the regulation of pantothenic acid in kidneys in a sex-specific manner. Adipic acid and its byproduct α-amino adipic acid are xenobiotic metabolites and their accumulation can bring about systemic complications due to their impact on mitochondrial toxicity. Adipic acid and its derivatives have been demonstrated to be toxic to both in vitro and in vivo mammalian model systems when administered in excess (Garthwaite & Regan 1980, Karlson et al. 1982, Ishikawa & Mine 1983, Huck et al. 1984, Estaras et al. 2020). A metabolomic study performed on 188 subjects identified adipic acid as the crucial indicator for developing type 2 diabetes (Wang et al. 2013). Along with being a marker for type 2 diabetes and hyperglycemia-induced toxicity, α-amino adipic acid is demonstrated to be a major marker and risk factor for cardiometabolic disorder (Lee et al. 2019). In a study in which dehydrogenase E1 and transketolase domain-containing 1 were knocked down in human myelogenous leukemia cell lines, the authors observed an increase in α-amino adipic acid levels (Wang et al. 2021). Increased α-amino adipic acid levels were associated with deteriorated mitochondrial function as measured by oxygen consumption rate using the Seahorse Mitostress assay (Wang et al. 2021). The oxidative phosphorylation protein subunit’s expression was reduced significantly with changes in mitochondrial morphology as demonstrated by distorted cristae (Wang et al. 2021). These changes were accompanied by a compensatory increase in mitochondrial area, mitochondrial DNA, and potential (Wang et al. 2021). Eventually, this increase in potential could lead to disruption of redox homeostasis.

α-amino adipic acid has been demonstrated to induce calcium imbalance, followed by reactive oxygen species induction, followed by oxidation of protein and lipid molecules in mouse pancreatic acinar cells (Estaras, 2020 #115). α-amino adipic acid derivative, 2 amino adipic acid has been demonstrated to be a major marker for aging, sepsis, and protein oxidation (Sell, 2007 #114). Bacterial species, probiotic Lactobacillus reuteri PL503 when exposed to α-amino adipic acid drastically enhanced reactive oxygen species generation and promoted protein carbonylation. All these evidence demonstrate the ability of α-amino adipic acid to act as an oxidative stress inducer across multiple species.

We are the first to report the elevation of adipic acid and its derivative α-amino adipic acid levels following chronic binge ethanol drinking in females. Along with adipic acid and its derivative, the female binge drinking group also demonstrated an increase in formylkynurenine, a kynurenine metabolite and hydroxytryptophan breakdown product. An elevation in kynurenine or its derivative formylkynurenine has been demonstrated to induce systemic and renal toxicity (Pawlak et al. 2001, Sallee et al. 2014, Debnath et al. 2017, Addi et al. 2018, Beier et al. 2020). In a study in mice, kynurenine 3-monooxygenase was demonstrated to be a major regulator of ischemic reperfusion injury in the kidney (Zheng et al. 2019). Knockout of kynurenine 3-monooxyngase has been associated with a reduction in inflammation and apoptosis-induced injury (Zheng et al. 2019). In humans, increased urinary secretion of kynurenic acid is considered a biomarker for acute kidney injury (Aregger et al. 2018). In rat striatal slices, 3-hydroxykynurenine was able to regulate cellular redox potential in a dose-dependent manner (Colin-Gonzalez et al. 2014). In a mouse model of intracerebral hemorrhage, kynurenic, through the mediation of aryl hydrocarbon, caused an enhancement in reactive oxygen species, along with disrupting mitochondrial redox balance and influencing neuronal function (Cuartero et al. 2014). We present the novel observation that formyl...
kynurenine, a kynurenine derivative, is increased by chronic binge drinking in a sex-specific manner and could be an ideal marker for binge drinking-induced early renal metabolomic changes in females.

Summary and conclusion

Our findings demonstrate that in both males and females binge drinking could influence mitochondrial function and redox homeostasis using two different metabolic pathways or clusters. In males, pantothetic acid- CoA axis and riboflavin-glutathione axis (Graphical abstract) downregulation following chronic binge drinking could dampen effective mitochondrial substrate metabolism due to lack of CoA and CoA adducts to metabolites and reduce cellular antioxidant capacity due to reduced glutathione reduction. This could accompany reduced mitochondrial oxidative function, reduction in active glutathione generation, and finally lead to redox imbalance. In females, chronic binge drinking caused an upregulation of adipic acid and its derivative α-aminoadipic acid and kynurenine derivative formyl-kynurenine. Both α-aminoadipic acid and formylkynurenine could enhance free radical generation in the form of reactive oxygen species, alter redox homeostasis, and bring about mitochondrial instability (Graphical abstract) (Pawlak et al. 2001, Wang et al. 2013, Colin-Gonzalez et al. 2014, Battelli et al. 2016, Lee et al. 2019, Zheng et al. 2019). Finally, uric acid has been a unanimous risk factor in both sexes following binge drinking (Graphical abstract). This work highlights the role of uric acid as a potential biomarker for binge drinking-induced renal injury independent of the sex-specific effects. Based on the available evidence presented in this work and from other’s work and mostly based on animal models, females are prone to higher risk in comparison to their male binge drinkers. One of the major reasons for the observed risk in female binge drinkers is due to their enhanced ethanol consumption in response to their male counterparts (Fig. 1). Previous reports have demonstrated and dissected the mechanisms behind this enhanced consumption and is mostly attributed to the contribution by female sex hormones and their respective receptors in the central regions. Our work validates the connection that enhanced ethanol consumption in females is accompanied by upregulation of oxidative stress markers that are sex or gender dependent and independent which could potentially lead to a disturbed renal redox homeostasis. Overall, our work has identified the altered metabolites which could contribute toward oxidative stress and if we are able to normalize these different metabolites and their derivatives in both males and females, we could limit chronic binge drinking-induced renal and systemic injury and restore redox balance and metabolic health. Our study will be a milestone in revealing alcohol binge drinking-induced changes in renal metabolomic landscape in a sex-specific manner and will aid in the advancement of others’ work in the field.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/REM-23-0005.

Declaration of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

DR, LMDC, and MS conducted experiments and obtained data and edited manuscript; KH, VN, and ML conducted experiments. CB helped with statistical analysis. AWL conceived the experimental design and edited the manuscript. PP conceived hypothesis, conducted experiments, collected data, wrote, and edited the manuscript and was primarily involved in plotting the figures. All the authors have read and approved the manuscript.

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References

Akhibge RE, Oladipo AA, Oyedokun PA, Hamed MA, Okeleji LO & Ajayi AF 2022 Upregulation of uric acid production and caspase 3 signalling mediates rohypnol-induced cardioirenal damage. Cardiovascular Toxicology 22 419–435. (https://doi.org/10.1007/s12012-022-09723-z)
Alam MM, Iqbal S & Naseem I 2015 Ameliorative effect of riboflavin on hyperglycemia, oxidative stress and DNA damage in type-2 diabetic


Gilep AA, Sushko TA & Usanov SA 2011 At the crossroads of steroid hormone biosynthesis: the role, substrate specificity and evolutionary development of CYP17. *Biochimica et Biophysica Acta* 1814 200–209. (https://doi.org/10.1016/j.bbapap.2010.06.012)


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