Research Paper

Sex-specific effect of ethanol on colonic short-chain fatty acids and derivatives in the mouse model using targeted LC/MS

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Abstract Ethanol consumption affects the human body, particularly the digestive system, leading to gut dysbiosis and dysregulation of metabolites. Although previous studies have focused on the effects of ethanol on liver metabolism, colon-specific metabolites have not been well explored. In this study, we investigated the effects of ethanol on colon-specific short-chain fatty acids (SCFAs) and their derivatives in a mouse model using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Colonic flush samples obtained from ethanol-fed (EF) and pair-fed (PF) mice of each sex were subjected to targeted analysis of SCFAs and SCFA esters of hydroxy fatty acids (SFAHFA) using LC-MS/MS. The levels of acetic acid (AA), propanoic acid (PA), and butyric acid (BA) were significantly increased in the male mouse EF group compared to those in the PF group. In contrast, the levels of AA and PA were decreased in female mice in the EF group compared to those in the PF group. Furthermore, the levels of AA-derived SFAHFAs were decreased in the male EF group but increased in the female EF group compared to those in the control group. Compared to the control groups, hydroxy fatty acid levels were significantly decreased in the EF groups of both sexes. Overall, ethanol exerted diverse effects on colon-specific metabolites in both male and female mice. This study provides new insights into sex-dependent metabolic changes associated with alcohol-induced gut microbiota dysfunction and their potential health impacts.

Key words: ethanol, short-chain fatty acids, SFAHFA, liquid chromatography, mass spectrometry, gut-microbial metabolites

1. Introduction

Alcohol is a globally prevalent psychoactive substance deeply rooted in various cultures that poses significant health risks. Alcohol was ranked as the seventh leading cause of death globally and contributed to 2.8 million deaths in 2016[1)]. The harmful effects of alcohol on the digestive system are closely related to the gut−liver axis, in which the enterohepatic circulation is essential for transporting products from the gut to the liver[2)]. Alcohol disrupts the gut−liver axis, altering the microbial composition and gut barrier integrity[3)], which leads to impaired nutrient absorption and contributes to conditions such as alcoholic hepatitis and cirrhosis. Dysbiosis disrupts tight junction proteins, allowing the translocation of particles into portal circulation[4)]. Tight junction maintenance is facilitated by short-chain fatty acids (SCFAs), which are byproducts of anaerobic fermentation by the food fibers[5)]. Fecal metabolome analysis of patients with alcohol use disorders showed a decrease in SCFAs, which could be explained by dysbiosis-impacting bacteria that produce SCFAs, including *Fae-*

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calibacterium[6].

Recent findings revealed a novel class of derivative lipids of SCFAs, namely SCFA esters of hydroxy fatty acids (SFAHFAs), which are abundant in the gut[7]. Gut microbiota is significant in the formation of these lipids, which are present in the colon and cecum[8]. Understanding the effect of alcohol on colonic-specific SCFAs and their derivatives was significantly advanced when chemically generated SFAHFAs were measured in rat intestinal contents and fecal samples[9].

Although existing research has explored lipid metabolism in alcohol-induced gut dysbiosis, notable gaps exist in our understanding of how ethanol specifically affects colonic SCFAs and SFAHFAs. A previous study used gas chromatography−mass spectrometry (MS) for analyzing human stool samples and demonstrated that excessive alcohol consumption disrupts the intestinal microbiota and causes dysbiosis, thus highlighting importance of fatty acids (FAs) in intestinal health and linking microbiota dysfunction to alcohol-related pathologies[10]. Furthermore, using liquid chromatography coupled with triple quadrupole and quadrupole time-of-flight MS, previous studies revealed that ethanol alters lipid metabolism by influencing the gut microbiota[11,12].

However, the existing literature on lipid metabolism in ethanol-induced gut dysbiosis is limited, and the sex-specific effects of ethanol on gut lipid metabolism remain unclear. Therefore, using liquid chromatography-tandem MS (LC-MS/MS), we investigated the sex-specific effects of ethanol on gut lipid metabolism in a mouse model. Unveiling the intricate connections between alcohol consumption and SCFA/SFAHFA variations, our findings contribute to a nuanced understanding of the mechanisms, offering the potential for sex-specific therapeutic strategies against alcohol-related pathologies.

2. Materials and Methods

2.1. Materials

LC/MS-grade solvents acetonitrile, methanol, and isopropanol, 1 M aqueous ammonium acetate, and acetic acid (AA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The reagents triethylamine (TEA), N,N-Dimethylethylenediamine (DMED), and acetic acid-d4 (AA-d4) and HPLC-grade chloroform were acquired from Sigma-Aldrich (St. Louis, MO, USA). Pentanoic acid-d9 (PA-d9) and palmitic acid-d31 were obtained from Cayman Chemical (Ann Arbor, MI, USA). Solid-phase extraction cartridges (MonoSpin C18-AX) were provided by GL Science (Tokyo, Japan). 2-Chloro-1-methylpyridinium iodide (CMPI), along with propionic acid (PA), butyric acid (BA), valeric acid (VA), and caproic acid (CA), were sourced from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The SFAHFA standards were synthesized in-house as per the protocols described in our previous study[9].

2.2. Animal samples

Male and female C57BL6 mice (8−10 weeks old) were bred at the institutional core facility after obtaining approval from The Institutional Animal Care and Use Committee at the University of Tennessee Health Science Center. Bothe male and female mice were fed a Lieber−DiCarli liquid diet (D710260, Dyets Inc., Bethlehem, PA, USA) for four weeks with ethanol (0% for two days, 1% for two days, 2% for two days, 4% for one week, 5% for one week, and 6% for one week) or pair-fed (PF) isocaloric maltodextrin (BioServ, Flemington, NJ, USA). At the end of the four-week feeding period, both groups of mice were euthanized, and the colonic contents were flushed with 3 mL of 0.9% saline. Flushed samples were frozen until lipid analysis. All experiments were performed in compliance with the relevant laws and/or institutional guidelines.

2.3. Extraction and targeted analysis of SCFA by LC-MS/MS

The extraction and analysis of SCFA were performed using the method developed in our previous studies[13]. Initially, 80 μL of acetonitrile was added to 20 μL of colonic flush, followed by the addition of 100 μL of 20 μM AA-d4 and 100 μL of 20 μM VA-d9 as internal standards. The mixture was then vortexed for 3 min at 3500 rpm and then centrifuged for 10 min at 15000 rpm and 4°C. The supernatant was carefully transferred into a new microtube, and derivatization was initiated by adding 20 μL of TEA and 10 μL of CMPI of 2 mM each. The mixture was vortexed for 3 min at 3500 rpm, following which 20 μL of 2 mM DMED was added to the microtube; the sample was further vortexed for 30 min at 3500 rpm and centrifuged (10 min, 15000 rpm, 4° C). The centrifugate was transferred to an LC vial, and 5 μL of this solution was injected into the LC-MS system.

Targeted analysis was performed using a Prominence ultrafast liquid chromatography (UFLC; Shimadzu, Kyoto, Japan) connected to a TSQ Quantum Mass Spectrometer

System (Thermo Fisher Scientific Inc., San Jose, CA, USA) operated in the positive-ion mode. The chromatographic separation was performed on a Hypersil GOLD C8 column (50 mm × 2.1 mm), which is a reversed-phase-type column with particle and pore sizes of 1.9 μm and 175 Å, respectively. The column oven was maintained at 40°C throughout the analysis. The mobile phase comprised a dual-solvent system: solvent A was Milli-Q water containing 20 mM ammonium acetate, and solvent B was a mixture of methanol and acetonitrile (50:50, v/v). The flow rate was set to 0.3 mL/min. The gradient program was initiated with solvent B at 100% for the first 1.5 min and held constant for 2 min. At 2.1 min, the proportion of solvent B was adjusted to 50% and maintained for 7 min. Subsequently, solvent B was returned to 100% after 7.5 min and maintained for 10 min. The electrospray ionization (ESI) source parameters were as follows: the spray voltage was 3,500 V; the sheath gas was 40 arbitrary units; the auxiliary gas was 25 arbitrary units; the capillary temperature was 250°C; and the HESI vaporizer temperature was 150°C. The SCFA concentrations were calculated from the linearity curves constructed using Xcalibur 2.2 software (Thermo Fisher Scientific., San Jose, CA, USA).

2.4. *Extraction and targeted analysis of SFAHFAs by LC-MS/MS*

The extraction and analysis of SFAHFAs were performed using a method developed in our previous studies[9]. Approximately 50 μL of colonic flush from ethanol-fed (EF) mice samples were transferred to Eppendorf tubes. To this, 100 μL of an internal standard mixture in methanol was added, which comprised 5 μM deuterated SFAHFA standard mixture (PAHPA-d30, BAHPA-d30, VAHPA-d30, and HAHPA-d30). In addition, 50 μL of methanol and 400 μL of Milli-Q water were added to the mixture. The resulting mixture was vortexed (3500 rpm, 1 min) and centrifuged (15,000 rpm, 1 min, 4°C) to collect the supernatant for solid-phase extraction using the MonoSpin C18-AX cartridges. The detailed workflow for the solid-phase extraction for SFAHFAs is described in our previous study[9].

Lipidomic analysis was conducted using a TSQ Quantum Access MAX Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled with the Prominence UFLC system (Shimadzu, Kyoto, Japan) operating under ESI in negative mode. The LC-MS conditions closely adhered to the methodology established in our previous research[9]. The elution solvents consisted of 10 mM ammonium acetate (pump A), isopropanol (pump B), and methanol (pump C). An Atlantis T3 C18 column (50 mm × 2.1 mm, 3 μm, Waters, Milford, USA) was used for the chromatographic separation, which was maintained at 40°C with a flow rate of 300 μL/min. The elution gradient was set as follows: 40% B and 20% C (0−7 min), 80% B and 10% C (7−14 min), 30% B and 35% C (14−18 min), and returned to the initial conditions. For each injection, the sample volume was set to 10 μL.

2.5. *Statistical analysis*

Data were visualized using Microsoft Excel 2021 and GraphPad Prism version 8.0.1. Statistical analysis involved Student's t-test with $p<0.05$, which was considered significant.

3. Results

Fig. 1 shows the outcomes of SCFA analysis of the colon contents of EF and PF mice. The concentration of each acquired SCFA level is shown in the bar graphs. Male PF and EF mice exhibited lower SCFA levels than female mice. In particular, AA and PA levels significantly decreased in the EF group of females, whereas the trend was reversed in males. Conversely, BA and VA levels increased in male and female mice in the EF group, respectively. However, no significant differences were observed in CA levels between sexes.

The concentrations of SFAHFAs detected in colonic flush samples are shown in Fig. 2 (A−C). The overall SFAHFA concentrations were higher in males than in females. In particular, the levels of the AA ester of 2-hydroxy palmitic acid (2-AAHPA), AA ester of 2-hydroxy arachidic acid (2-AAHAA), AA ester of 2-hydroxy behenic acid (2-AAHBA), AA ester of 2-hydroxy tricosylic acid (2-AAHTA), AA ester of 2-hydroxy lignoceric acid (2-AAHLA), and AA ester of 2-hydroxy pentacosylic acid (2-AAHPSA) exhibited significant increases in the EF group of females compared to those in the PF group, whereas a reversed trend was observed in males. Furthermore, the AA ester of 2-hydroxy heneicosylic acid (2-AAHHA), BA ester of 2-hydroxy behenic acid (2-BAHBA), PA ester of 2-hydroxy cerotic acid (2-PAHCA), BA ester of 2-hydroxy cerotic acid (2-BAHCA), CA ester of 2-hydroxy cerotic acid (2-CAHCA), BA ester of 2-hydroxy lignoceric acid (2-BAHLA), and PA ester of 2-hydroxy pentacosylic acid

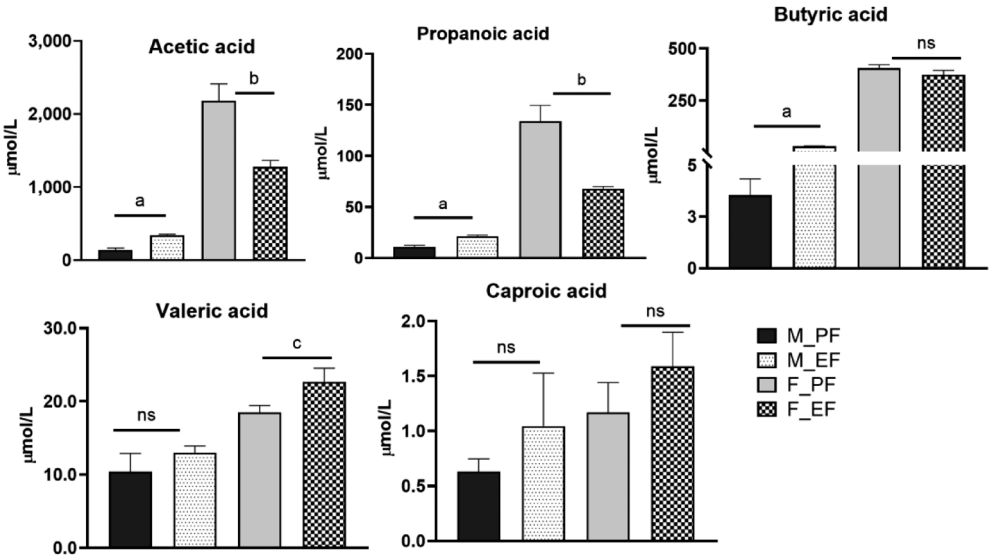

Fig. 1. Amount of SCFAs in colon contents of PF and EF groups of male and female mice.
(M_PF: Male pair-fed, M_EF: Male ethanol-fed, F_PF: female pair-fed, F_EF: female ethanol-fed, n=4 for each group). Student's t-test: $p<0.001$ (a), $p<0.01$ (b), $p<0.05$ (c), and ns: non-significant.

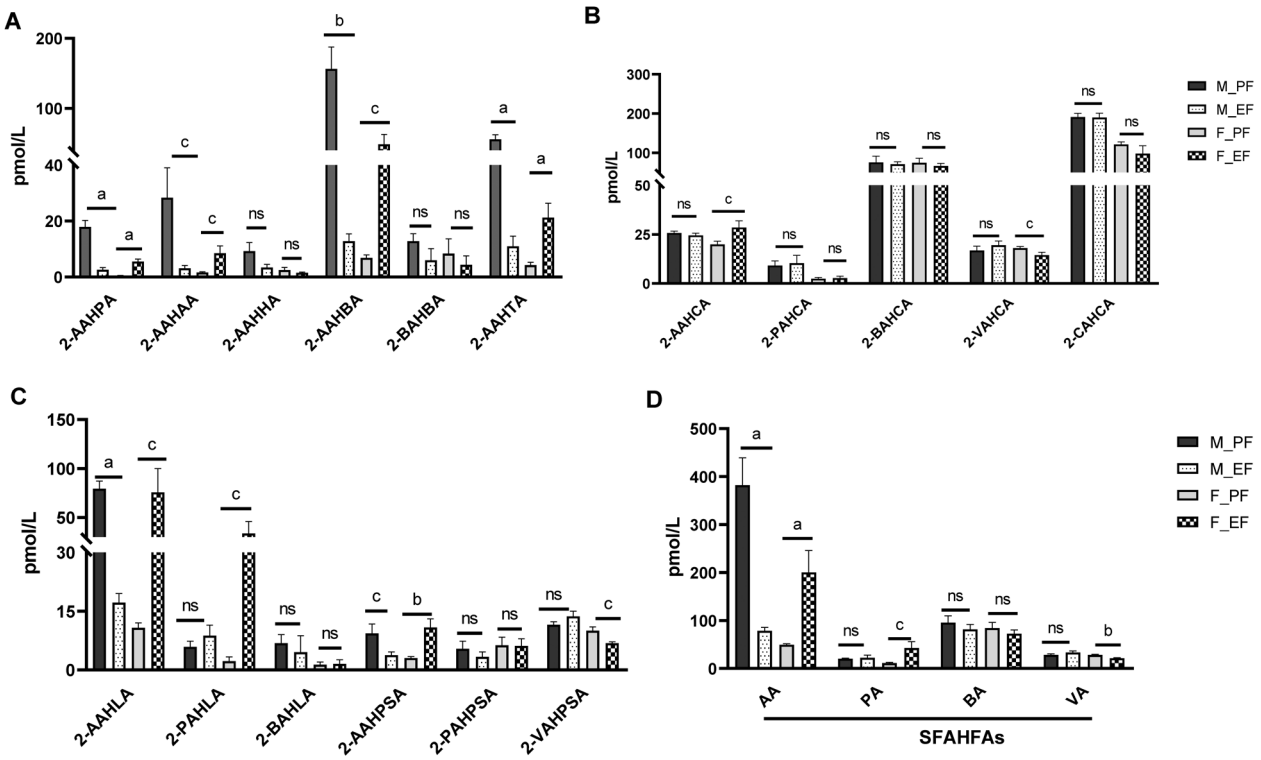

Fig. 2. Amount of SFAHFAs detected in colon contents of the PF and EF groups of male and female mice.
The concentrations of SFAHFAs at the molecular level are shown in A, B, and C, and the total SFAHFAs of each SCFA-derived lipids are shown in D. (M_PF: Male pair-fed, M_EF: Male ethanol-fed, F_PF: female pair-fed, F_EF: female ethanol-fed, n=4 for each group). Student's t-test: $p<0.001$ (a), $p<0.01$ (b), $p<0.05$ (c), and ns: non-significant.

(2-PAHPSA) did not show any significance between the EF and PF groups of sexes.

The levels of the AA ester of 2-hydroxy cerotic acid (2-AAHCA) and PA ester 2-hydroxy lignoceric acid (2-PAHLA) were significantly increased in female EF mice whereas no such differences were observed in male EF mice. However, the levels of the VA ester of 2-hydroxy cerotic acid (2-VAHCA) and VA ester of 2-hydroxy pentacosylic acid (2-VAHPSA) were significantly decreased in female EF mice, but no significant difference was observed

in male mice. Fig. 2(D) shows the total amount of SFAHFAs based on their SCFA acyl chains in the PF and EF mice of both sexes. In female mice, the levels of AA-derived SFAHFAs were significantly increased in the EF group, whereas the opposite trend was observed for the male mice. However, BA showed no significant differences between sexes. The levels of PA-derived SFAHFAs were significantly increased in the female EF group, whereas those of VA-derived SFAHFAs decreased. The concentrations of hydroxy FAs (HFAs) in PF and EF mice of both sexes are shown in Fig. 3. Notably, higher concentrations of HFAs were observed for females than males except for 2-hydroxy palmitic acid (2-HPA) in both the PF and EF groups. A significant decrease in HFA concentration was observed in female mice with EF. However, no significant variations were observed for HFA concentrations in male EF mice, except for 2-HPA and 2-hydroxy behenic acid (2-HBA), which were significantly decreased in the EF group. The concentrations of all the SCFA, SFAHFA, and HFA analyzed in the colonic flush samples of PF and EF mice were provided in Table 1. The data shows a large deviation in the concentration levels among each animal, suggesting that additional experiments with a large animal number would be essential to draw any further conclusions.

4. Discussions

Alcohol-induced dysbiosis has a detrimental effect on gut integrity, leading to the development of acute (alcoholic hepatitis) and chronic (alcohol-related cirrhosis) liver disorders. Many metabolites such as SCFAs are produced by the anaerobic gut microbiota as byproducts of the fermentation of dietary fibers[14,15] and have been demonstrated to have multiple favorable effects on mammalian energy metabolism[16]. Therefore, metabolites (such as SCFAs) produced by beneficial bacteria in the gut aid absorption and utilization. These metabolites also support mucosal immunity, preserve intestinal homeostasis, and shield the intestinal mucosal barrier.

As shown in Fig. 1, a significant decrease in the SCFA concentration was observed for the female EF mice compared to female PF mice. The relevance of these findings is further supported by a study on the fecal metabolome in humans with alcohol use disorders. This independent study revealed a reduction in SCFA levels, which is consistent with our results. The observed decrease in SCFAs is likely attributable, in part, to dysbiosis, which negatively affects SCFA-producing bacteria, namely *Faecalibacterium*[6,10]. The observed increase in SCFA levels in male EF mice may be attributed to inherent sex-specific hormonal and physiological differences. This is in agreement with a recent study on Chinese bamboo rats, which showed significant differences in microbial composition and genetic/metabolic pathways between female and male rats despite shared captive and feeding conditions[17]. These sex-specific variations may contribute to unique responses to alcohol exposure, influencing SCFA levels. The congruence between our mouse model results and the insights from the bamboo rat study highlights a robust association between ethanol consumption, gut dysbiosis, and alterations in SCFA levels in a sex-specific manner. Furthermore, parallel findings across different species enhance our understanding of the intricate relationships between alcohol consumption, gut microbiota, and downstream physiological effects.

The modulation of SFAHFAs in the PF and EF groups of both sexes is detailed in Fig. 2. Previous investigations into SFAHFAs have indicated a decline in their levels in the

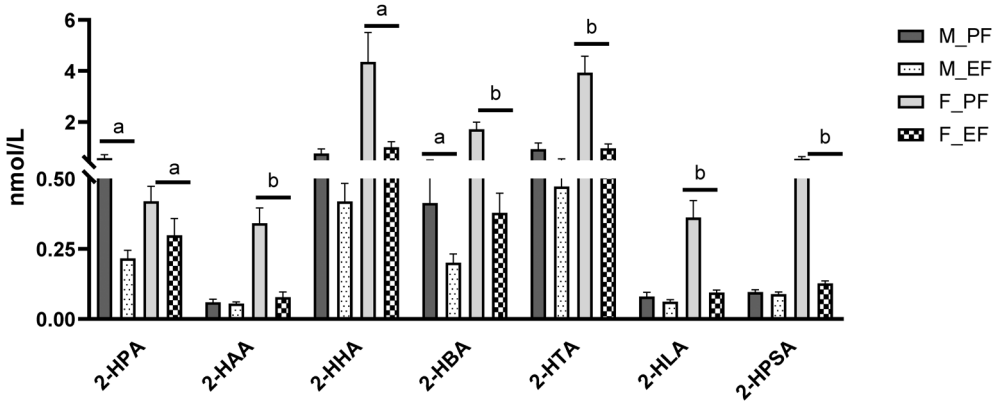

Fig. 3. Amount of hydroxy fatty acids detected in the PF and EF groups of male and female mice.
(M_PF: Male pair-fed, M_EF: Male ethanol-fed, F_PF: female pair-fed, F_EF: female ethanol-fed). Student's t-test: $p<0.001$ (a) and $p<0.01$ (b).

Table 1. The concentrations of short-chain fatty acids, SFAHFAs, and hydroxy fatty acids analyzed in the colonic flush samples

	Male mice								Female mice							
	PF1	PF2	PF3	PF4	EF1	EF2	EF3	EF4	PF1	PF2	PF3	PF4	EF1	EF2	EF3	EF4
SCFA	Concentration (μmol/L)															
Acetic acid	170.27	179.21	141.17	72.10	347.47	359.45	303.28	361.51	1938.33	1781.13	2181.98	2822.14	1205.66	1526.45	1266.54	1112.41
Propanoic acid	12.49	13.89	9.47	7.98	22.05	23.56	21.44	18.05	113.18	118.68	123.34	180.34	72.94	69.97	63.74	65.13
Butyric acid	4.52	4.27	4.21	1.15	28.96	35.08	26.96	32.18	381.42	375.36	428.11	440.38	388.65	410.53	384.06	309.70
Valeric acid	12.41	15.56	9.97	3.67	12.65	14.94	10.60	13.81	16.82	18.53	21.19	17.47	22.38	23.92	26.68	17.78
Caproic acid	0.32	0.83	0.59	0.78	1.24	0.28	2.33	0.33	0.51	1.48	0.95	1.73	1.87	1.75	2.05	0.68
SFAHFA	Concentration (pmol/L)															
2-AAHPA	13.14	16.03	18.49	24.00	0.78	4.00	3.62	1.99	0.87	0.00	0.00	0.56	3.94	5.77	7.77	4.59
2-AAHAA	9.86	13.74	33.51	56.24	4.31	0.59	4.91	2.68	1.90	1.20	0.87	2.33	3.69	4.58	10.88	14.77
2-AAHHA	4.69	3.27	14.75	14.28	5.58	2.59	0.62	4.80	2.48	3.41	0.00	4.09	0.93	2.16	1.08	1.81
2-AAHBA	82.97	125.93	206.27	210.27	18.07	5.62	12.66	14.81	4.11	8.23	6.56	8.57	31.37	18.15	70.79	74.44
2-BAHBA	14.76	19.52	8.33	8.45	0.00	0.00	6.20	17.58	23.43	7.32	0.00	2.62	2.39	13.73	0.00	1.29
2-AAHTA	53.74	40.22	56.92	71.75	8.25	3.45	11.69	20.53	5.33	6.45	1.70	3.43	19.71	7.34	29.85	28.01
2-AAHCA	24.07	24.41	27.96	26.50	23.91	21.59	25.06	27.32	17.04	17.26	22.09	23.29	26.82	38.15	27.33	21.54
2-PAHCA	4.93	15.74	8.32	7.66	4.33	22.30	8.36	6.18	1.74	1.76	4.32	1.80	3.10	0.00	4.94	2.89
2-BAHCA	59.86	40.31	94.74	108.01	77.16	56.25	83.23	67.73	67.42	78.72	47.28	104.35	76.47	78.12	50.83	60.93
2-VAHCA	11.10	15.50	20.02	20.39	20.21	20.60	23.68	13.51	17.03	16.63	18.31	20.12	18.54	14.51	13.28	11.31
2-CAHCA	200.01	190.97	207.92	165.63	200.45	208.75	191.88	158.62	135.42	112.72	109.77	128.03	109.25	150.49	68.24	63.15
2-AAHLA	86.57	58.90	75.62	95.98	20.05	13.44	22.17	13.00	11.16	13.41	11.19	7.28	36.32	30.99	115.21	119.97
2-PAHLA	9.73	4.99	2.63	6.17	13.49	13.10	3.13	5.24	0.50	5.43	2.12	0.94	31.76	7.71	66.58	28.26
2-BAHLA	8.80	1.34	5.80	11.45	0.00	0.88	17.23	0.00	1.37	0.00	3.27	0.69	0.00	0.00	4.29	1.95
2-AAHPSA	4.69	10.92	6.39	15.34	2.21	5.40	4.91	2.64	3.23	2.64	2.22	4.01	7.38	6.74	15.12	14.14
2-PAHPSA	7.44	0.54	9.40	4.28	2.17	2.28	7.06	1.90	12.05	6.17	4.45	2.55	3.13	4.61	5.24	11.52
2-VAHPSA	10.54	13.67	11.44	10.61	13.28	12.37	17.55	11.49	7.23	11.35	10.07	11.41	5.81	7.49	7.00	7.06
AA_SFAHFA	279.72	293.42	439.91	514.35	83.16	56.67	85.63	87.77	46.12	52.59	44.63	53.55	130.16	113.89	278.04	279.26
PA_SFAHFA	22.10	21.26	20.35	18.11	20.00	37.68	18.55	13.33	14.29	13.35	10.89	5.29	37.99	12.32	76.76	42.67
BA_SFAHFA	83.43	61.17	108.87	127.91	77.16	57.13	106.66	85.31	92.22	86.05	50.55	107.66	78.86	91.85	55.13	64.17
VA_SFAHFA	21.64	29.17	31.46	31.00	33.49	32.97	41.23	25.00	24.25	27.98	28.38	31.53	24.35	22.00	20.28	18.37
HFA	Concentration (nmol/L)															
2-HPA	0.48	0.40	0.48	0.98	0.23	0.15	0.20	0.28	0.29	0.49	0.52	0.38	0.21	0.18	0.41	0.39
2-HAA	0.07	0.04	0.04	0.08	0.07	0.04	0.05	0.06	0.20	0.41	0.44	0.31	0.06	0.04	0.10	0.12
2-HHA	0.74	0.58	0.50	1.27	0.60	0.30	0.36	0.42	1.90	6.79	5.78	2.95	0.66	0.59	1.39	1.38
2-HBA	0.43	0.30	0.23	0.69	0.28	0.13	0.19	0.20	1.04	2.13	2.19	1.50	0.31	0.21	0.47	0.51
2-HTA	0.96	0.66	0.54	1.60	0.68	0.28	0.44	0.49	2.33	4.87	5.11	3.41	0.80	0.58	1.20	1.29
2-HLA	0.08	0.06	0.05	0.12	0.08	0.05	0.05	0.07	0.21	0.45	0.47	0.32	0.09	0.07	0.11	0.11
2-HPSA	0.11	0.09	0.08	0.11	0.10	0.07	0.09	0.10	0.33	0.66	0.71	0.53	0.14	0.10	0.14	0.12

colons of rats subjected to a high-fat diet, in contrast to an increase observed in mice infected with the influenza virus[7,18]. The observed downregulation of specific SFAHFAs derived from PA and BA in antibiotic-treated mouse fecal samples underscores their specificity for the gut microbiota[12]. Previous studies have consistently demonstrated that supplementation with SCFAs, achieved through interventions such as a high-fiber diet or probiotics, enhanced gut epithelial integrity and mitigated liver injury in alcoholic models[19,20,21]. Considering the apparent association between SFAHFAs and SCFAs, these two biomolecules are reasonably correlated. This consistency strongly supports the continued exploration of interventions involving SCFAs and SFAHFAs as promising strategies for addressing pathologies associated with alcohol consumption.

A notable decrease in the HFA concentration in female EF mice is shown in Fig. 3. In male EF mice, except for 2-HPA and 2-HBA, no significant alterations were observed. As the generation of HFAs is linked with the abundance of free FAs (FFAs), these HFA results are in agreement with previous research that highlighted the pivotal role of FFA metabolism in the progression of alcoholic liver disease[22]. The implications of FFAs in alcohol-induced gut dysbiosis, characterized by microbiota imbalances and potential contribution to gastrointestinal disorders, have been accentuated in numerous studies[23]. An animal study has suggested that maintaining optimal intestinal FFA levels, particularly saturated fatty acids, can enhance and preserve the integrity of the intestinal gut barrier[24]. The observed decline in HFA concentration after ethanol consumption in our study adds to this understanding by emphasizing the intricate interplay between alcohol consumption, FA metabolism, and gut health. These findings reinforce the importance of targeted interventions to modulate HFAs, particularly in maintaining intestinal barrier integrity, as a potential strategy for addressing alcohol-related pathologies. The findings of the study are summarized

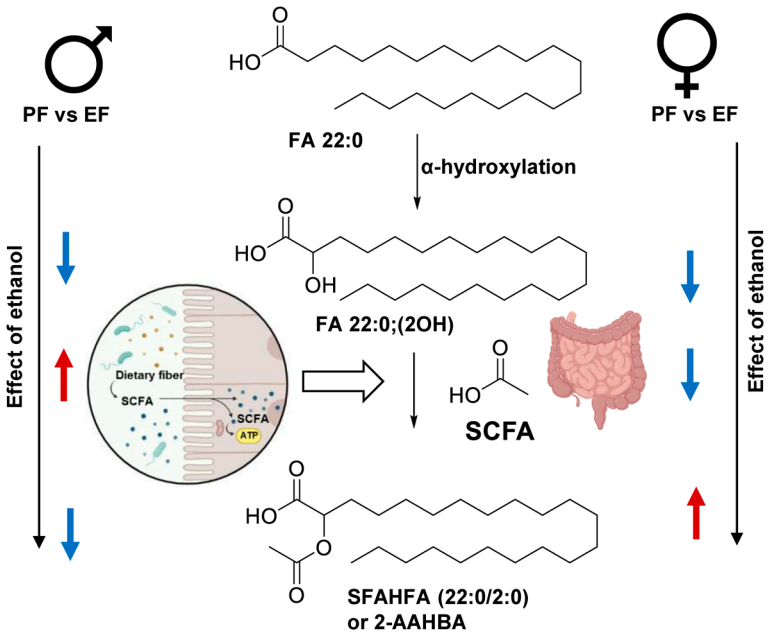

Fig. 4. Summary of changes in the precursor (FA 22:0(2OH), AA) and product (2-AAHBA) SFAHFA metabolism.

in Fig. 4 using a representative FA and its metabolites. The concentration of precursor FA 22:0(2OH) was decreased in the EF group of both sexes, and that of AA was increased in the male EF mice but decreased in female EF mice. However, an opposite trend was observed for 2-AAHBA in the EF groups of both sexes. This study has some limitations, including the mechanisms underlying the changes in the reported metabolites, which have not been explored. However, this study provides the first insight into the effects of ethanol on colonic metabolites produced by the gut microbiota.

In conclusion, our investigation into the effect of ethanol on colon-specific metabolites in a mouse model revealed distinct sex-dependent metabolic changes. These findings shed light on the intricate and diverse effects of alcohol-induced changes in gut microbiota, highlighting the importance of considering sex-specific responses to understand the potential health impacts of microbial metabolites.

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Conflict of interest

The authors declare no competing financial interest.

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