Low-Dose Ethanol Has Impacts on Plasma Levels of Metabolites Relating to Chronic Disease Risk in SAMP8 mice

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Summary The effects of low-dose alcohol on experimental animals are unclear. This study examined plasma metabolites in senescence-accelerated mice 8 (SAMP8) given low-dose ethanol, and compared them with aging progress and skeletal muscle strength. Male SAMP8 mice (10-wk-old) were given drinking water containing 0% (control), 1%, 2%, or 5% (v/v) ethanol for 14 wk. Compared with the control group, only mice who consumed 1% ethanol experienced a lower senescence score at 18 and 23 wk, as well as an increased limb grip strength at 21 wk. Plasma metabolites of control, 1% and 2% ethanol groups were analyzed by capillary electrophoresis–time-of-flight mass spectrometry (CE-TOF/MS). Among the 7 metabolites affected by ethanol, noteworthy is the positive association of the ethanol levels in drinking water with the levels of α -ketoglutarate (antioxidant and anti-inflammatory metabolite) and hippurate (antioxidant and microbial co-metabolite) ($p < 0.05$). Intriguingly, the levels of 2-hydroxyisobutyrate (the biomarker of energy metabolism and microbial co-metabolite) were higher in the 1% ethanol group ($p < 0.05$), but not in the 2% ethanol group as compared to the control. Furthermore, the levels of some of the metabolites affected were correlated with some variables in the grading score of senescence and muscle strength. This study provides a novel insight into how low-dose ethanol in SAMP8 mice modulates the levels of circulating metabolites relating to chronic disease risk.

Key Words low-dose ethanol, senescence-accelerated mice, antioxidant, anti-inflammatory metabolite, microbial co-metabolite

In 1991, Marmot and Brunner (1) reported a U- or J-shaped relationship between alcohol (i.e., ethanol) consumption and disease risk: moderate consumption was associated with lower risks of chronic diseases such as cardiovascular and cancer, whereas high consumption was associated with a higher risk. Interestingly, minimal (or low dose) consumption has been reported by several epidemiological studies to have a beneficial effect on overall health (2, 3). However, there are epidemiological studies to report no beneficial effects of low-dose alcohol (4, 5). Thus, the effects of low-dose alcohol remain controversial.

Given the limitations of epidemiological studies, the direct effects of low-dose ethanol on health cannot be confirmed and, thus, remain controversial. In humans, laboratory investigations evaluating the impact of alcohol on an individual's overall health is challenging be-

cause of confounding factors such as socioeconomic status, ethnicity, medical history, and frequency and type of alcoholic beverages consumed. Therefore, it is necessary to conduct animal experiments to clarify the effects of low-dose ethanol. Martin et al. (6) reported that rats consuming water with 1% ethanol experienced a reduction in total cholesterol and triglyceride levels, thereby decreasing the risk of cardiovascular disease. Osaki et al. (7) found that consumption of 1% ethanol in drinking water ($\sim 0.3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) improved liver function in rats fed a high-fat diet, whereas intake of 2% ethanol had only a minor effect. Kimoto et al. (8) subsequently reported that in senescence-accelerated mice 8 (SAMP8), being widely used in brain aging study, consumption of 1% ethanol suppressed senescence grading scores and markedly increased brain mRNA levels of alcohol dehydrogenase 1 (an enzyme responsible for the degradation of ethanol and lipid peroxidation products), whereas 2% ethanol does not. Moreover, in our recent study, we found that consumption of 1% ethanol in drinking water ($0.4\text{--}0.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) suppressed colon tumorigenesis and lowered the intestinal level of *Clostridium leptum* (immunosuppressor) in rats administered 1,2-dimethylhydrazine,

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Abbreviations: CE, capillary electrophoresis; CE-TOF/MS, capillary electrophoresis–time-of-flight mass spectrometry; DMG, N,N-dimethylglycine; ESI, electrospray ionization; 2-HIB, 2-hydroxyisobutyrate; IBD, inflammatory bowel disease; SAMP8, senescence-accelerated mice 8.

whereas 2% ethanol did not (9). The results of another previous study suggest that low ethanol consumption ($0.7 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) protects against cerebral ischemia/reperfusion injury by suppressing postischemic inflammation via upregulation of cystathionine γ -lyase that, in turn, produces hydrogen sulfide (an anti-inflammatory and antioxidant metabolite at low concentrations) (10). Thus, these experimental studies suggest that intake of low-dose alcohol has beneficial effects on health. In contrast, there is some evidence that low-dose ethanol consumption leads to adverse effects in experimental animals. Melchior et al. have reported that low doses of ethanol (0.5 or $1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) impair working memory in mice (11). Moreover, Sakazaki et al. have reported that low-dose ethanol ($\sim 1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) aggravates allergic dermatitis in mice (12).

Given that the effects of low-dose ethanol are controversial and incompletely understood, our primary aim was to analyze the levels of plasma metabolites in SAMP8 mice administered low-dose ethanol by capillary electrophoresis–time-of-flight mass spectrometry (CE-TOF/MS) in the present study. As a secondary aim, we examined the correlations between the levels of metabolites and senescence and muscle strength in SAMP8 mice, based on preliminary discovery showing low-dose ethanol (1% ethanol in drinking water) increases limb grip strength (Izu et al., unpublished results), although high-dose ethanol is reported to cause skeletal muscle damage or alcoholic myopathy through mechanisms involving oxidative stress (13).

MATERIALS AND METHODS

Animal experiments. Five-week-old male SAMP8 mice (Japan SLC, Shizuoka, Japan) were kept in plastic cages (5 mice per cage) in a controlled environment (temperature $22 \pm 2^\circ\text{C}$, lights on from 00:00 to 12:00). Mice were given commercial food pellets (MF, Oriental Yeast, Tokyo, Japan) and water ad libitum. After 6 wk of acclimation, the mice were divided into 4 groups ($n=10$ per group) given deionized water containing 0% (control), 1%, 2%, or 5% (v/v) ethanol ad libitum for 14 wk. Body weight was measured once every 2 wk, and food and drinking water intake were measured twice per week. On the day of dissection, food and drinking water were removed from 8:00 h. The mice were sacrificed (between 13:00 and 15:00) by decapitation after anesthesia with 3–4% isoflurane gas. All procedures followed the Guide for the Care and Use of Laboratory Animals (National Research Institute of Brewing, Animal Care Committee) and were approved by the Animal Care Committee of the National Research Institute of Brewing, Japan (Ethical approval No. 28-1).

Senescence grading. A grading system (8) was used to quantify and evaluate the degree of senescence. This system comprises 11 items that can be divided into 4 categories: behaviors (reactivity and passivity), skin and hair (glossiness, coarseness, hair loss, and skin ulcers), eyes (periophthalmic lesions, cataract, corneal ulcer, and corneal opacity), and spine (lordokyphosis). Each item was scored weekly by careful inspection. The total

grading score was the sum of all 11 items scores.

Grip strength test. A grip strength test was performed from 10:00–12:00 h by using a grip strength meter tester (GPM-100B, Melquest, Toyama, Japan) to quantify the muscular strength of the limbs and forearms of mice (14). Briefly, each mouse was lifted by its tail and moved horizontally toward the grip bar (used to test the forearms) or grid (used to test all 4 limbs) of the meter until the animals' front legs or all 4 limbs reached and firmly grabbed the testing grip or grid, respectively. The peak force strength of the forearms or all limbs was measured and recorded while the mouse was slowly pulled from the meter at a constant speed.

Plasma CE-TOF/MS analysis. During dissection, blood was collected from the control group (0% ethanol), 1% ethanol, and 2% ethanol groups ($n=8$ per group) into EDTA-containing tubes (JIS T3233, Becton, Dickinson and Company, Fukushima, Japan). Plasma was obtained by centrifugation at $1,300 \times g$ for 15 min. Owing to financial limitations, 2 plasma samples of equal volume within the same group were randomly pooled into a single sample for metabolomics analysis. Thus, a total of 12 plasma samples (4 pooled plasma samples each from the control, 1% ethanol, and 2% ethanol groups) were subjected to metabolomics analysis (Human Metabolome Technologies Inc., Tsuruoka, Japan) according to the manufacturer's instructions (15). Plasma samples in the 5% ethanol group were not analyzed because there appeared to be no notable difference in the data of total senescence score and grip strength between the 2% and 5% ethanol groups. Briefly, $50 \mu\text{L}$ serum sample was mixed into $450 \mu\text{L}$ adjusted methanol in order to obtain $10 \mu\text{M}$ internal standard. Then, $500 \mu\text{L}$ chloroform and $200 \mu\text{L}$ Milli-Q water were added and mixed, followed by centrifugation at $2,300 \times g$ at 4°C for 5 min. The supernatant was ultrafiltered using a 5-kDa centrifugal filter unit (Millipore, Billerica, MA, USA) at $9,100 \times g$ at 4°C for 120 min, followed by evaporation to dryness. Metabolomics analysis was performed using a capillary electrophoresis–time-of-flight mass spectrometry (CE-TOF/MS) system (Agilent Technologies, Waldbronn, Germany), and separation was performed in fused-silica capillaries ($50 \mu\text{m}$ i.d. $\times$ 80 cm total length). For cationic metabolites, the MS conditions were as follows: sample injection, pressure injection, 50 mbar for 10 s; CE voltage: positive, 27 kV; ionization: electrospray ionization (ESI) in positive mode; capillary voltage: 4,000 V; scan range: m/z 50–1,000. For anionic metabolites, the MS conditions were as follows: sample injection, pressure injection, 50 mbar for 10 s; CE voltage: positive, 30 kV; ionization: ESI in negative mode; capillary voltage: 3,500 V; scan range: m/z 50–1,000.

Statistical analysis. Data were analyzed by Dunnett's test. The significant level was set at $p < 0.05$. Correlation was analyzed by measure of Pearson's correlation coefficient.

Table 1. Body weight, food intake, fluid intake, liver weight, and skeletal muscle weight of SAMP8 mice.

	Ethanol			
	0% (Control)	1%	2%	5%
Final body wt (g)	32.8±0.8 ¹	34.7±1.9	33.8±1.2	32.6±0.9
Gains in body wt (g for 14 wk)	5.1±0.3	6.6±1.0	6.3±0.8	4.5±0.5
Liver (g)	1.58±0.05	1.48±0.09	1.54±0.06	1.51±0.05
Gastrocnemius muscle (g)	0.144±0.007	0.143±0.005	0.136±0.005	0.148±0.006
Total food intake (g for 14 wk)	540 ²	497	530	475
Total fluid intake (g for 14 wk)	757	663	817	637
Ethanol intake (g for 14 wk)	0	5.3	13.1	25.5
Ethanol intake (g·kg body weight ⁻¹ ·d ⁻¹)				
at 14 wk-old	0	1.38	3.92	7.50
at 18 wk-old	0	1.16	3.11	5.91
at 22 wk-old	0	1.19	2.29	5.62

¹ Values are mean±SE ($n=10$), and unaffected by ethanol intake ($p>0.05$, Dunnett's test).

² Because the 5 mice were kept together in a cage, the average of the 2 cages data of food intake, fluid intake and ethanol intake in each group (10 mice per group) was indicated.

RESULTS

Growth, food intake, and fluid intake

Final body weight, food intake, fluid intake, liver weight, and gastrocnemius weight were unaffected by ethanol consumption ($p>0.05$; Table 1). Based on fluid intake and body weight data, daily ethanol ingestion for the 1%, 2%, and 5% ethanol groups was approximately 1.2–1.4, 2.3–3.9, and 5.6–7.5 g·kg⁻¹·d⁻¹, respectively (Table 1).

Senescence grading scores

The senescence grading scores of total, skin and hair, and spine categories at 18 and 23 wk are shown in Fig. 1. Compared to the control group (0% ethanol in water), the 1% ethanol group had significantly lower total (Fig. 1A), skin and hair (Fig. 1B), and spine (Fig. 1C) senescence grading scores (Dunnett's test, $p<0.05$) at 18 and 23 wk, whereas the 5% ethanol group had significantly lower spine senescence grading scores at these time points (Dunnett's test, $p<0.05$). Consumption of 2% ethanol caused no effects on these scores. The senescence grading scores of the behavior and eyes categories were unaffected by ethanol consumption ($p>0.05$, data not shown). Overall, the 1% ethanol group exhibited larger reductions in senescence grading scores than the 2% and 5% ethanol groups.

Limb and forearm grip strength

Compared with the control group, only the 1% ethanol group had an 18% greater limb grip strength at 21 wk (Dunnett's test, $p<0.05$; Fig. 2A). There was no effect of ethanol consumption on the limb grip strength at 17 wk and on the forearm grip strength at 17 and 21 wk ($p>0.05$; Fig. 2B). The total senescence score at 23 wk was not correlated with the limb and forearm grip strength at 21 wk ($r=-0.356$ and -0.451 , respectively, $p>0.05$).

Plasma metabolites

Peaks for 185 metabolites were detected, and concen-

Fig. 1. Effects of ethanol intake on senescence grading scores in SAMP8 mice. A, total scores; B, scores of skin and hair; C, scores of spine. Values are mean±SE ($n=10$). *Significantly different from the control (0% ethanol) (Dunnett's test, $p<0.05$).

trations for 71 of them were quantified with or without using the standards. Levels of 7 metabolites were significantly affected by ethanol consumption (Dunnett's test, $p<0.05$; Fig. 3). Meanwhile, ethanol consumption tended to affect the levels of other 7 metabolites ($0.05<$

$p < 0.10$; Supplemental Online Material, Table S1).

Anti-oxidative metabolites: α -ketoglutarate, hippurate, pipecolate, and isethionate

Compared with the control group, the levels of α -ketoglutarate were significantly (32%) higher in the 2% ethanol group ($p < 0.05$, Fig. 3A) but not in the 1% eth-

anol group ($p < 0.05$). Hippurate levels were significantly greater in the 1% and 2% ethanol groups than the control group (37% and 69% higher, respectively, $p < 0.05$; Fig. 3B). The levels of pipecolate were significantly increased by 2% ethanol (19% higher, $p < 0.05$; Fig. 3C). The levels of lysine, a precursor of pipecolate, were not affected by ethanol consumption ($p > 0.10$, data not shown). Isethionate levels showed a trend of increasing with ethanol intake ($0.5 < p < 0.1$; Supplemental Online Material, Table S1). The levels of other antioxidant metabolites such as carnosine, homocarnosine, anserine, glutathione, and taurine were unaffected ($p > 0.10$, data not shown). In addition, the levels of α -ketoglutarate related metabolites, such as citrate, *cis*-aconitate, isocitrate, succinate, and malate were also unaffected ($p > 0.10$, data not shown). The concentrations of ethanol in drinking water were significantly correlated with the levels of α -ketoglutarate ($r = 0.768$), hippurate ($r = 0.923$), pipecolate ($r = 0.664$), and isethionate ($r = 0.678$) ($p < 0.05$ for all).

Methyl donors: betaine, *N,N*-dimethylglycine (DMG), and methionine

The levels of betaine were significantly lower in the 1% and 2% ethanol groups than the control group (23% and 25% lower, respectively, $p < 0.05$; Fig. 3D). Compared to the control group, DMG level was significantly lower in the 2% ethanol group (18% lower; $p < 0.05$) but not the 1% ethanol group ($p > 0.05$, Fig. 3E). The levels of ethanol in drinking water were significantly correlated with the levels of betaine ($r = -0.642$) and DMG ($r = -0.776$) ($p < 0.05$ for each). The level of another methyl donor, methionine, tended to be lower in the 1% and 2% ethanol groups than the control group (both 16% lower; $0.05 < p < 0.1$; Supplemental

Fig. 2. Effects of ethanol intake on grip strength in SAMP8 mice. A, limbs; B, forearms. Values are mean $\pm$ SE ($n = 10$). * Significantly different from the control (0% ethanol) (Dunnett's test, $p < 0.05$).

Fig. 3. Effects of ethanol intake on plasma levels of α -ketoglutarate, hippurate, pipecolate, betaine, *N,N*-dimethylglycine (DMG), 2-hydroxyisobutyrate (2-HIB), and ethanolamine. A, α -ketoglutarate; B, hippurate; C, pipecolate; D, betaine; E, DMG; F, 2-HIB; G, ethanolamine. Values are mean $\pm$ SE ($n = 4$). Metabolites were quantified according to the relative peak area of each analyte to the internal standard obtained from CE-TOF/MS. * Significantly different from the control (0% ethanol) (Dunnett's test, $p < 0.05$).

Fig. 4. Correlations of plasma metabolites with total senescence score, grip strength of limbs, and grip strength of forearms. A, betaine vs. total senescence score at 23 wk; B, betaine vs. grip strength of limbs at 21 wk; C, DMG vs. grip strength of limbs at 21 wk; D, 2-HIB vs. grip strength of forearms at 21 wk; E, ethanolamine vs. grip strength of limbs at 21 wk. Metabolites were quantified according to the relative peak area of each analyte to the internal standard obtained from CE-TOF/MS. White circle, control (0% ethanol); black circle, 1% ethanol; white triangle, 2% ethanol.

Online Material, Table S1). Other methyl donors and their related metabolites, such as sarcosine, choline, glycine, serine, glycerophosphocholine, and phosphorylcholine were not affected ($p > 0.1$, data not shown).

2-Hydroxyisobutyrate (2-HIB)

The level of 2-HIB was significantly higher in the 1% ethanol group than that in the control and 2% ethanol groups (30% and 23% higher, respectively; $p < 0.05$). The levels of ethanol in drinking water were not correlated with the level of 2-HIB ($r = 0.134$, $p > 0.05$, Fig. 3F).

Ethanolamine

Levels of ethanolamine in both the 1% and 2% ethanol groups were slightly lower than that in the control group (13% and 18% lower, respectively; $p < 0.05$, Fig. 3G). The level of ethanol in drinking water was significantly correlated with the level of ethanolamine ($r = -0.820$, $p < 0.05$).

Other metabolites

Ethanol treatment also tended to affect the levels of other metabolites such as phenacetate, glycerate, homovanillate, kynurenine, and N^6 -methyllysine ($0.05 < p < 0.10$; Supplemental Online Material, Table S1).

Correlations of plasma metabolite levels with senescence scores and grip strength

The plasma levels of betaine were positively correlated with the total senescence grading score at 23 wk ($r = 0.641$, $p < 0.05$; Fig. 4A). Meanwhile, the levels of betaine, DMG, and ethanolamine were inversely correlated with the limb grip strength at 21 wk ($r =$

-0.711 , -0.618 , and -0.608 , respectively, $p < 0.05$; Fig. 4B, 4C, and 4E). The levels of 2-HIB were significantly correlated with the forearm grip strength at 21 wk ($r = 0.627$, $p < 0.05$; Fig. 4D), but weakly associated with limb grip strength at 21 wk ($r = 0.513$, $p = 0.088$) or total senescence score at 23 wk ($r = -0.534$, $p = 0.073$).

DISCUSSION

Consistent with findings from our previous study (8), the total senescence grading score in the 1% ethanol group was the lowest among the four treatment groups, whereas the scores of the 2% and 5% ethanol groups did not differ from those of the control. Furthermore, the present study revealed that, when compared against the control group, only mice given 1% ethanol had greater limb grip strength at 21 wk. This data suggests that 1% ethanol may be favorable for senescence and muscle strength, but not 2% and 5% ethanol, thereby supporting the J-curve hypothesis of alcohol. Of note, we previously reported that 2% ethanol stimulated spontaneous locomotor movement in SAMP8 mice, but 1% ethanol did not (8); thus, the enhanced limb grip strength observed in mice given 1% ethanol is likely to be unrelated.

The present study indicated consumption of ethanol was positively associated with the levels of antioxidants, α -ketoglutarate and hippurate. Emerging evidence suggests that α -ketoglutarate, an intermediary metabolite in the Krebs cycle, can be used in the therapy of several

diseases, given its immunomodulatory, antioxidant, anti-inflammatory, and anticancer effects (16, 17). Recent studies further suggest that α -ketoglutarate can prolong lifespan (18). α -Ketoglutarate is involved in various fundamental processes, including collagen synthesis (19) and epigenetic regulation (20). α -Ketoglutarate also influences several age-related processes, including stem cell proliferation (21) and osteoporosis (22). Accordingly, it is possible that low-dose ethanol might delay processes related to aging in mice, although we did not detect any significant association between α -ketoglutarate and the total senescence score and muscle strength in our study.

Hippurate or *N*-benzoylglycine possesses radical-scavenging activity (23), and is a mammalian-microbial co-metabolite that originates from bacterial fermentation of dietary aromatic compounds (polyphenols, purines or aromatic amino acids) to benzoate which is further conjugated to glycine in the liver (24). Actually, the commercial pellets (MF, Oriental Yeast) used in the present study were made from corn, wheat bran, defatted soy bean, defatted rice bran, alfalfa, fish meal, defatted cow milk, soy bean oil, and beer yeast (Catalog of Oriental Yeast) and therefore contain polyphenols. There is growing evidence that hippurate is negatively associated with metabolic syndrome and Crohn's disease, and positively associated with brain health (25–29). Brial et al. found that chronic subcutaneous infusions of hippurate in mice fed a high-fat diet improved glycemic control and insulin secretion (through increased β -cell mass), as well as reduced hepatic inflammation and fibrosis (30). Collectively, the results imply that low-dose ethanol may have beneficial effect on aging mice by elevating hippurate. Recent studies suggest that low-dose ethanol inhibits oxidative stress via the upregulation of anti-oxidant systems such as cystathionine γ -lyase and alcohol dehydrogenase 1 (8, 10). Since low-dose ethanol elevated hippurate levels in our mice, it is presumable this would have a beneficial impact on the aforementioned conditions, warranting mechanistic studies. The levels of pipecolate was slightly, but significantly increased by 2% ethanol. Previous study suggested picolate can protect mammalian cells against oxidative stress in HEK293 cells through the mechanism involving pipecolate oxidase (31). However, other study indicated pipecolate induces oxidative stress in vitro in cerebral cortex of rats (32). Thus, at present, it is unknown if the increased pipecolate by 2% ethanol is beneficial or not.

With regards to the methyl donors, the levels of DMG decreased slightly following ethanol consumption, which corroborates with earlier, related research in humans (33). Another methyl-donor, betaine levels were also lower in the ethanol groups. Of note, the levels of betaine were positively associated with senescence score at 23 wk. Additionally, the levels of betaine and DMG were inversely correlated with limb grip strength at 21 wk. Importantly, studies suggest that dietary methionine restriction extends lifespan possibly through the restriction of methyl donors (34). Therefore, intake of

low-dose ethanol may be especially beneficial for aging mice, calling for further investigations. However, methyl donor deficiency is reported to have negative effects on homocysteine metabolism, intestinal differentiation and barrier function, and gene expression patterns (35). Thus, the significance of the modulation of plasma levels of methyl-donors in response to low-dose ethanol remains unclear, warranting further research.

Intriguingly, the present study showed that mice in the 1% ethanol group experienced significant increases in the levels of 2-HIB compared with those in the control and 2% ethanol groups. Furthermore, the levels of 2-HIB were significantly associated with forearm grip strength at 21 wk, and weakly associated with total senescence score at 23 wk. This metabolite is derived from the microbial degradation of valine (36), and two studies involving healthy, young men who are moderate drinkers showed that the levels of 2-HIB in urine elevated for the first 2 h after acute alcohol consumption, whereas, a study of young male athletes showed that same to be elevated after an 800-m run (37, 38). The levels of urinary 2-HIB in the active inflammatory bowel disease (IBD) patients were lower than those in healthy control subjects (39). 2-HIB is detected at high levels in the obese patients and undernourished mice (40, 41). On the other hand, a recent study suggests that histone 2-hydroxyisobutyration plays roles in glucose metabolism and aging in yeast (42). Furthermore, the extension effect of calorie restriction on yeast lifespan has been considered to be partly due to higher 2-hydroxyisobutyrylation of histone (42). Thus, emerging evidence points to 2-HIB as a biomarker of energy metabolism. As such, it is possible that consumption of 1% ethanol may be capable of modulating energy metabolism in mice. Since the response of 2-HIB to low-dose ethanol appears to be similar to the J-curve effect of alcohol, it is of interest to elucidate the implication of increased 2-HIB in the 1% ethanol group.

Mice given 1% or 2% ethanol exhibited a decrease in plasma ethanolamine levels, which correlated negatively with limb grip strength at 21 wk. Ethanolamine is known to stimulate the generation of phosphatidylethanolamines, membrane phospholipids that have a wide range of structural and functional properties, including lipid synthesis, mitochondrial biogenesis, and autophagy (43). Less is known about plasma ethanolamine, although it is suggested to be a positive marker of skeletal muscle turnover (44). Our study found no correlation between the weight of the gastrocnemius and ethanolamine level, and even a negative correlation between limb grip strength and plasma ethanolamine level, indicating more research is needed to confirm its impact, or lack thereof, on muscle tissue.

CONCLUSIONS

This study used CE-TOF/MS to show that low-dose ethanol increases plasma levels of the antioxidants α -ketoglutarate and hippurate, the anti-inflammatory metabolite α -ketoglutarate, and the marker of energy metabolism 2-HIB, collectively providing new insights

for minimizing chronic disease risk. Modulation of plasma hippurate and 2-HIB, microbial co-metabolites, by low-dose ethanol is of great interest since low-dose ethanol may affect the microbial co-metabolites through mechanisms involving microbiota. Further studies are necessary to elucidate how low-dose ethanol modulates the underlying biological mechanisms in order to confirm its efficacy in health promotion.

Authorship

Research conception and design: TK, HI, and NK; experiments: CF, YY, AK, and HI; statistical analysis of the data: CF, YY, and HI; interpretation of the data: CF, YY, AK, and NK; writing of the manuscript: CF, YY, TK, and NK; CF and YY were contributed equally to this work.

Disclosure of state of COI

The authors declare that they have no conflicts of interest.

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Supporting information

Supplemental online material is available on J-STAGE.

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