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ORIGINAL ARTICLE

Basic Study

Effects of ethanol and sex on propionate metabolism evaluated *via* a faster ^{13}C-propionate breath test in rats

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Abstract

BACKGROUND

Alcoholism is regarded as a risk factor for vitamin B_{12} (VB_{12}) deficiency. Because V B_{12} serves as a coenzyme of methylmalonyl-CoA mutase, a key enzyme in propionate metabolism, the ^{13}C-propionate breath test (PBT) has been studied as a non-invasive diagnostic modality for VB_{12} deficiency. However, the conventional PBT requires 2 h, which is inconvenient in clinical practice. We hypothesized that a faster PBT can be used to evaluate propionate metabolism and is more easily adaptable for clinical practice.

AIM

To evaluate a faster PBT for assessing the effects of long-term ethanol consumption on propionate metabolism in ethanol-fed rats (ERs).

METHODS

ERs were obtained by replacing standard drinking water (for control rats, CRs) with 16% ethanol solution in descendants of F344/DuCrj rats. Faster PBT was performed by administering ^{13}C-propionate aqueous solution to male and female ERs and CRs by inserting a metal tubule from the mouth to the stomach; exhaled gas was collected in a bag to measure its $^{13}CO_2/^{12}CO_2$ isotope ratio *via* infrared isotope spectrometry. Serum VB_{12} and alanine transaminase (ALT) levels were measured *via* chemiluminescence immunoassay and the lactate dehydrogenase-ultraviolet method, respectively. We evaluated statistical differences in mean body weight, change in $^{13}CO_2$ ($\Delta^{13}CO_2$‰), peak $\Delta^{13}CO_2$‰, and serum VB_{12} and ALT, between males and females and between ERs and CRs using the *t*-test and Mann-Whitney U test for normally and non-normally distributed variables, respectively.

RESULTS

Males weighed significantly more than females ($P < 0.001$); CRs weighed significantly more than ERs ($P < 0.008$). $\Delta^{13}CO_2$ reached a peak (C_{max}) at 20 min and

30 min in females and males, respectively, decreasing after 20-30 min without rebound in all groups. Males had significantly higher C_{max} and $\Delta^{13}CO_2$ at 15-45 min than females ($P < 0.05$; for all pairs). Propionate metabolism was enhanced in male ERs relative to male CRs, whereas metabolism did not differ markedly between ERs and CRs for females. Males had higher serum VB_{12} levels than females, without prominent differences between the ER and CR groups. Male CRs had notably higher ALT levels than male ERs. Thus, chronic ethanol consumption may trigger fatty acid production *via* intestinal bacteria and changes in gut microbiome composition.

CONCLUSION

Faster PBT shows that 16% ethanol consumption promotes propionate metabolism without inducing liver injury. This PBT may be used clinically to evaluate gut flora status.

Key Words: Alcoholism; Breath test; Carbon isotope; Gut flora; Propionate; Vitamin B12

Core Tip: Alcoholism is a risk factor for vitamin B_{12} (VB_{12}) deficiency. The ^{13}C-propionate breath test (PBT) is a diagnostic modality for VB_{12} deficiency, but requires 2 h for completion. We applied a faster PBT to evaluate propionate metabolism using an ethanol-fed rat model. After ^{13}C-propionate administration, the $^{13}CO_2/^{12}CO_2$ isotope ratio of gas collected every 5 min for 60 min was measured using infrared isotope spectrometry. The $\Delta^{13}CO_2$ peak occurred within 30 min. Ethanol-fed males showed marked propionate metabolism without associated liver injury. This study demonstrates the potential of the faster PBT to evaluate propionate metabolism under various clinical conditions.

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INTRODUCTION

Chronic alcoholism is a risk factor for vitamin B_{12} (VB_{12}) deficiency[1,2]. Because VB_{12} works as a coenzyme of methylmalonyl-CoA mutase, a key enzyme in propionate metabolism (Figure 1), the ^{13}C-propionate breath test (PBT) has been studied as a non-invasive diagnostic modality for VB_{12} deficiency, with favorable results[3,4]. For instance, Wagner *et al*[3] reported that the conventional PBT could reliably predict VB_{12} deficiency in humans, with an area under the curve of the receiver operating curve value of 0.88. Propionate is a ubiquitous short chain fatty acid produced by intestinal bacteria, such as *Phascolarctobacterium*[5]. Emerging evidence suggests that intestinal microbial flora have a healing influence on alcoholic liver damage[6], and propionate produced by intestinal bacteria has protective effects against alcoholic liver damage[7]. Thus, we believe that PBT may provide important information not only regarding VB_{12}deficiency, but also regarding alcohol metabolism and alcoholic liver damage.

However, the conventional PBT requires 2 h to complete, which can be highly inconvenient for patients in clinical settings. Thus, in the present study, we aimed to evaluate the potential of a faster PBT for assessing the effects of long-term ethanol consumption on propionate metabolism as well as VB_{12} deficiency using ethanol-fed rats (ERs) as an animal model of chronic alcoholism. As the protective effects of estrogen against VB_{12} deficiency have been reported[8], we also evaluated the effect of sex-related differences on propionate metabolism, as detected by the faster PBT.

MATERIALS AND METHODS

Animals and treatments

All animal experiments were performed with approval of the Toho University School of Medicine, No. 21-51-4960. Descendants of F344/DuCrj rats purchased from CLEA Japan Inc. (Tokyo, Japan) for our previous study[9] were used to establish the ER and control rat (CR) groups for this study. All rats used in the present study were 18th-generation descendants of the originally established ER and CR groups, maintaining the lines within treatments (*i.e.*, parents of ERs were ERs, parents of CRs were CRs).

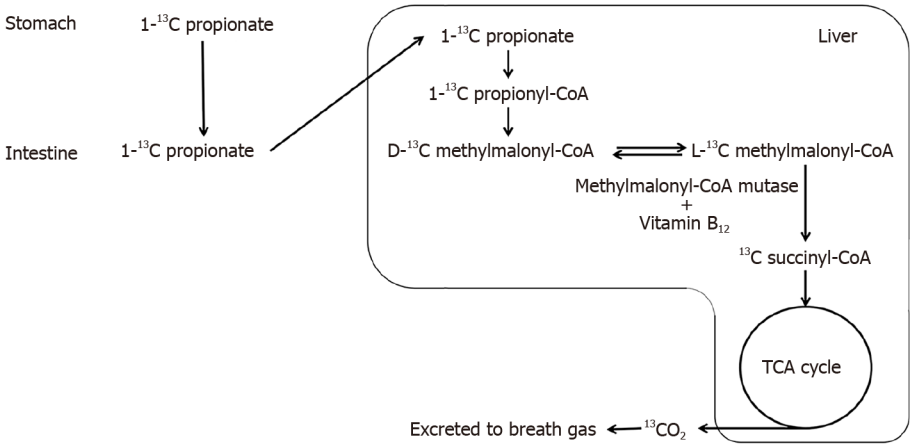

Figure 1 Propionate metabolism and the measuring principle of the propionate breath test. TCA: Tricarboxylic acid. Citation: Sasaki Y, Sato T, Maeda T, Komatsu F, Kawagoe N, Imai T, Shigeta T, Kashima N, Urita Y. [In Press] Evaluation of the One-Hour ^{13}C-Propionate Breath Test in 49 Patients from a Single Center in Japan to Detect Vitamin B_{12} Deficiency. *Med. Sci. Monit.* 2023 [DOI: 10.12659/MSM.940238]. Copyright © The Authors 2023. Published by Medical Science Monitor, International Scientific Information, Inc.

All rats were housed with their mothers until weaning at 4 wk of age. Subsequently, all rats were individually housed in a controlled environment (temperature, 23 ± 2 °C; humidity, 55% ± 5%) and provided a standard diet (CE-7; CLEA Japan Inc., Tokyo, Japan) and drinking liquid *ad libitum*. In the ER group, a 16% ethanol solution (Japanese Sake, Ozeki Corporation, Hyogo, Japan) was provided as a substitute for water by replacing the content of water bottles with ethanol solution in all cages of ERs.

A total of 16 ERs (8 males and 8 females) and 16 CRs (8 males and 8 females) aged 27-30 wk were used in the experiments; ERs continuously consumed alcohol for 23-27 wk. We used available descendants of F344/DuCrj rats that we had utilized in previous studies[9]. Therefore, we did not perform sample size calculation, randomization, or blinding.

Administration of ^{13}C-propionate and collection of exhaled gas

We purchased 1-^{13}C-sodium propionate from Cambridge Isotope Laboratories (Andover, MA, United States) and prepared a ^{13}C-propionate aqueous solution at 1 g/mL using distilled water immediately before administration. Body weight was measured immediately before administration. We performed gastrointestinal intubation in each rat and used a metal tubule, extending from the mouth to the stomach, to administer 0.1 mL/g of the ^{13}C-propionate solution. Immediately after administration, the rats were individually placed in the chambers of a dedicated exhaled-gas collection machine consisting of sealed chambers, pumps, and collecting bags, designed by Uchida *et al*[10]. We collected 100-200 mL of exhaled gas in the collecting bag for 90 s every 5 min for a total of 60 min.

Measurement of the ^{13}C recovery rate

Because ^{13}C-propionate is metabolized in the liver and exhaled as $^{13}CO_2$ (Figure 1), we measured the $^{13}CO_2/^{12}CO_2$ isotope ratio of the collected gas using infrared isotope spectrometry (POCone; Otsuka Electrics Co, Ltd., Hirakata, Japan) and monitored the change in $^{13}CO_2$ ($\Delta^{13}CO_2$‰), as reported previously[11].

Evaluation of serum VB_{12} and alanine transaminase levels

After collecting the exhaled gas for 60 min, the rats were immediately anesthetized *via* sevoflurane inhalation, and 5-10 mL of venous blood was collected from the inferior vena cava and the right atrium under laparotomy. After collecting sufficient blood samples, the animals were euthanized by rapid blood release. The blood was immediately centrifuged (relative centrifugal force: 1700 ×*g*) for 10 min, and the serum was collected. The serum was promptly frozen and submitted to FUJIFILM VET Systems Co. Ltd. (Tokyo, Japan) for measuring serum VB_{12} and ALT levels *via* chemiluminescence immunoassay and the lactate dehydrogenase-ultraviolet method, respectively.

Statistical analyses

We analyzed $\Delta^{13}CO_2$‰ measured every 5 min after ^{13}C-propionate administration for 60 min and serum VB_{12} levels, comparing the sexes and the ER and CR groups. The normality of the distribution of all variables was evaluated using the Kolmogorov-Smirnov test, and differences between groups were compared using the *t*-test and Mann-Whitney U test for normally and non-normally distributed variables, respectively. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using Stata/IC software (version 15.1; Stata Corp., College Station, TX, United States). We used R 4.2.0 for construction of graphics[12]. The statistical methods were reviewed by Yosuke Sasaki from the Toho

University School of Medicine (the first author). As Yosuke Sasaki has completed several certified biostatistics courses, we did not obtain additional biostatical review suggestions by external biomedical statisticians.

RESULTS

Influence of ethanol and sex on body weight

Body weight was significantly higher ($P < 0.0001$) in males (335.8 ± 37.0 g) than in females (176.6 ± 21.9 g). In addition, body weight was significantly higher in CRs than in ERs for both males and females ($P = 0.0082$ and $P = 0.005$, respectively, Table 1).

Sex-related difference in PBT results

The $\Delta^{13}CO_2$ reached its peak (C_{max}) at 20 min and 30 min in females and males, respectively. The $\Delta^{13}CO_2$ decreased after 20-30 min without rebound in both groups (Table 2 and Figure 2). Therefore, the overall trends in $\Delta^{13}CO_2$ over time were similar between males and females, although C_{max} was delayed in males and $\Delta^{13}CO_2$ was significantly higher in males at 30 min and thereafter (Table 2 and Figure 2). The C_{max} and $\Delta^{13}CO_2$ values between 15 and 45 min were significantly higher in males than in females ($P < 0.05$, Table 2). Considering these sex-based differences, we further compared the effects of ethanol in males and females separately.

Effects of ethanol on propionate metabolism

Propionate metabolism was accelerated in the ERs relative to that in the CRs in males (Figure 3), with $\Delta^{13}CO_2$ at 10 and 20 min being markedly higher in male ERs. However, $\Delta^{13}CO_2$ after 40 min was higher in the CR group (Table 3). The $\Delta^{13}CO_2$ reached C_{max} earlier in the ERs (at 20 min) than in the CRs (at 30 min). These findings suggest that ethanol promoted propionate metabolism in male rats. However, propionate metabolism was similar between the ER and CR groups among females, without any significant differences ($P > 0.110$ for all pairs, Table 3, Figure 4).

Effects of ethanol on serum VB_{12} and ALT levels

The serum VB_{12} levels were significantly higher in males than in females ($P = 0.0013$, Table 4); however, no significant differences were observed between the ER and CR groups for either sex ($P > 0.05$ for all pairs, Table 4). In contrast, serum ALT levels were significantly higher in male CRs than in male ERs ($P = 0.0347$, Table 5).

DISCUSSION

In this study, we compared propionate metabolism using a faster PBT in rats and compared serum VB_{12} and ALT levels between males and females and between ER and CR groups. Overall, our study demonstrates that (1) The faster PBT is useful for evaluating differences in propionate metabolism after administration of a ^{13}C-propionate solution; (2) Males show greater propionate metabolism, with higher serum VB_{12} levels, than females; (3) Ethanol consumption promotes propionate metabolism in male rats only; and (4) Ethanol consumption reduces body weight and serum ALT levels.

In the faster PBT, $\Delta^{13}CO_2$ peaked at 30 min, then decreased over time without rebound in all groups. Accordingly, we consider the faster PBT, which is completed within only 60 min after ^{13}C propionate administration, to be sufficiently sensitive to evaluate propionate metabolism, as a substitute for the conventional PBT that requires collecting exhaled gas for 2 h.

Using the PBT, our study showed a higher C_{max} and $\Delta^{13}CO_2$ between 15 and 45 min in male rats than in female rats, which suggests that male rats have stronger propionate metabolism. Suppression of carbohydrate metabolism and promotion of lipid metabolism by estrogen in females have been proposed as mechanisms contributing to lower carbohydrate metabolism in females than in males[13]. Furthermore, a protective effect of estrogen against VB_{12} deficiency in fertile females has been reported, along with higher susceptibility to VB_{12} deficiency in postmenopausal women[8,14]. Considering that VB_{12} works as a coenzyme of methylmalonyl-CoA mutase, and that serum VB_{12} levels were not pathologically low in the rats used in our study, we postulate that the lower propionate metabolism detected by the faster PBT and the lower serum VB_{12} levels in females than in males may reflect underlying physiological sex-related differences in carbohydrate metabolism associated with estrogen.

As we aimed to use the faster PBT to evaluate impaired propionate metabolism due to VB_{12} deficiency and liver disease caused by chronic alcohol consumption, we expected to find lower propionate metabolism and higher serum ALT levels in the ER group than in the CR group. However, we obtained contrasting results, with acceleration of propionate metabolism in the ER group and higher serum ALT levels in the CR group. Changes in the gut flora caused by chronic alcohol consumption may explain the promotion of propionate metabolism in the ER group. Using male marmosets, Zhu *et al*[15] reported

Table 1 Body weight (g) in the ethanol-fed rat and control rat groups

	ER (*n* = 16)	CR (*n* = 16)	*P* value
Male (*n* = 16)	313.0 (18.4)	358.5 (37.5)	0.0082[a]
Female (*n* = 16)	162.5 (14.9)	190.6 (18.7)	0.005[a]

[a]*P* < 0.05.

Data are presented as mean (SD). CR: Control rat; ER: Ethanol-fed rat.

Table 2 Propionate breath test results in male and female rats

	Male (*n* = 16)	Female (*n* = 16)	*P* value
C_{max}	1478.0	1302.3	0.039[a]
5 min	381.8	412.5	0.358
10 min	838.6	837.4	0.983
15 min	1259.2	1114.1	0.049[a]
20 min	1455.7	1238.2	0.011[a]
25 min	1471.3	1267.9	0.007[a]
30 min	1418.5	1248.2	0.008[a]
35 min	1330.6	1197.1	0.010[a]
40 min	1240.3	1136.5	0.013[a]
45 min	1134.5	1075.9	0.035[a]
50 min	1044.7	994.6	0.050
55 min	959.9	918.8	0.083
60 min	884.2	845.6	0.309

[a]*P* < 0.05.

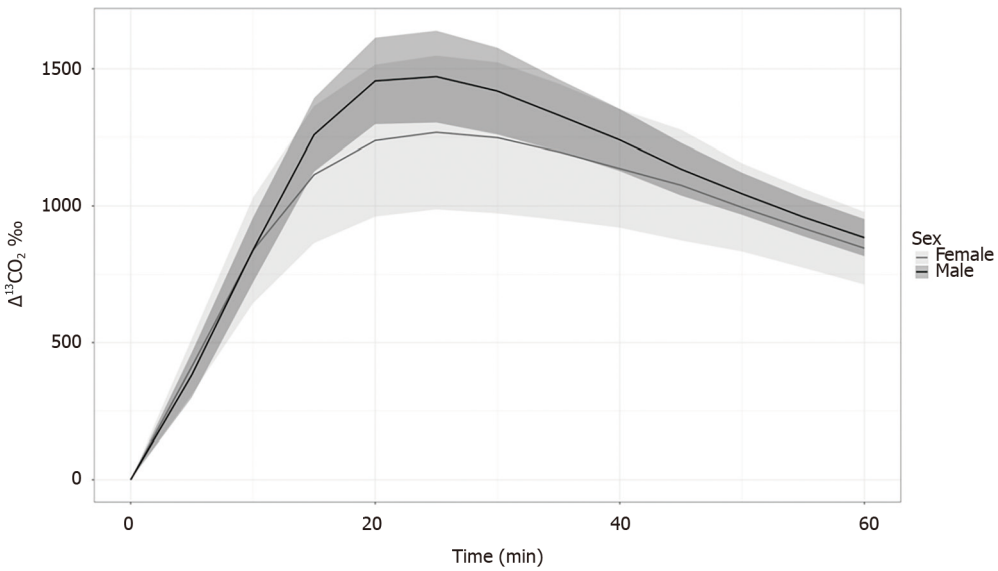

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Figure 2 Comparison of faster propionate breath test results between the male and female rats. Note: Tinted area around each line indicates the standard deviation range.

Table 3 Propionate breath test results in the ethanol-fed rat and control rat groups

	Male			Female		
	CR (*n* = 8)	ER (*n* = 8)	*P* value	CR (*n* = 8)	ER (*n* = 8)	*P* value
C_{max}	1422.8	1533.1	0.192	1366.1	1238.5	0.401
5 min	345.8	417.9	0.080	410.8	414.2	0.950
10 min	752.3	924.9	0.0008[a]	867.6	807.3	0.552
15 min	1186.1	1332.5	0.023[a]	1203.2	1025	0.160
20 min	1402.3	1509.1	0.186	1324.7	1151.7	0.223
25 min	1402.1	152.6	0.235	1334.5	1201.4	0.462
30 min	1379.9	1457	0.348	1296.8	1199.7	0.753
35 min	1313.9	46	0.629	1237.2	1157.1	0.753
40 min	1223.2	1257.3	0.565	1179.2	1093.8	0.529
45 min	1126.6	1142.3	0.756	1131.3	1020.6	0.208
50 min	1051.8	1037.5	0.724	1050.4	938.7	0.172
55 min	974.2	945.6	0.436	976.4	861.3	0.142
60 min	904.1	864.4	0.256	898.9	792.3	0.110

[a]$P < 0.05$.
CR: Control rat; ER: Ethanol-fed rat.

Table 4 Effects of ethanol and sex on serum vitamin B_{12} (pg/mL)

	Male (*n* = 16)	Female (*n* = 16)	*P* value[1]	Total (*n* = 32)
All (*n* = 32)	884.0 (124.8)	728.5 (123.3)	0.0013[a]	-
ER (*n* = 16)	878.0 (152.5)	696.3 (152.5)	0.0323[a]	787.1 (175.1)
CR (*n* = 16)	890.0 (100.2)	760.8 (81.7)	0.0134[a]	825.4 (110.7)
P value[2]	0.8551	0.3117	-	0.4658

[1]Comparison of males *vs* females.
[2]Comparison of Ethanol-fed rat *vs* Control rat.
[a]$P < 0.05$.
Data are presented as mean (SD). CR: Control rat; ER: Ethanol-fed rat.

Table 5 Serum alanine transaminase (IU/L) levels

	ER (*n* = 16)	CR (*n* = 16)	*P* value
Total (*n* = 32)	55.7 (16.1)	79.3 (39.5)	0.0347[a]
Male (*n* = 16)	65.0 (16.8)	110.3 (32.3)	0.0034[a]
Female (*n* = 16)	46.4 (8.5)	48.3 (9.9)	0.6898

[a]$P < 0.05$.
Data are presented as mean (SD). CR: Control rat; ER: Ethanol-fed rat.

that the concentrations of short-chain fatty acids, including propionate, depend on changes in intestinal bacteria, based on an observed reduction in fecal propionate levels along with a reduction in the relative abundance of *Phascolarctobacterium* in the gut. Moreover, Watanabe *et al*[5] reported that the substrates of short-chain fatty acids, including propionate, produced by intestinal bacteria depend not only on a single bacterial strain, but also on the specific composition of other bacteria present in the gut. According to these reports, ethanol can serve as both a potential substrate of fatty acid production by

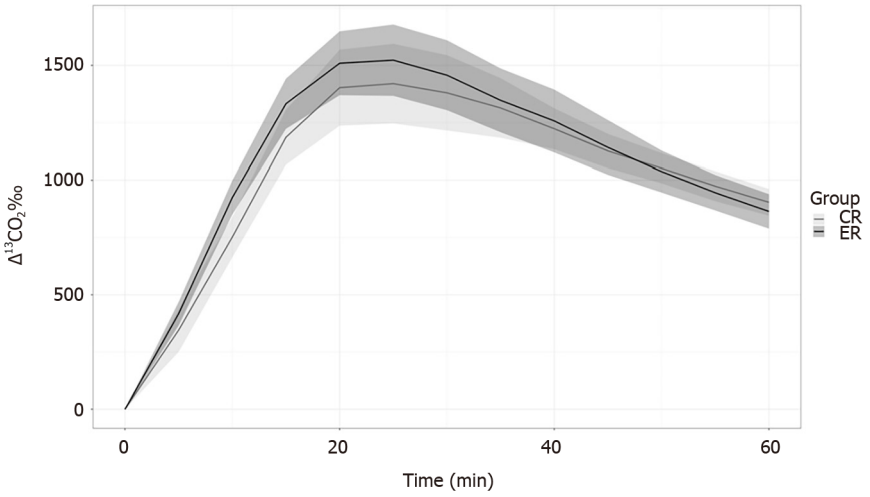

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Figure 3 Comparison of propionate breath test results between the male ethanol-fed rat and male control rat groups. Note: Tinted area around each line indicates the standard deviation range. CR: Control rat; ER: Ethanol-fed rat.

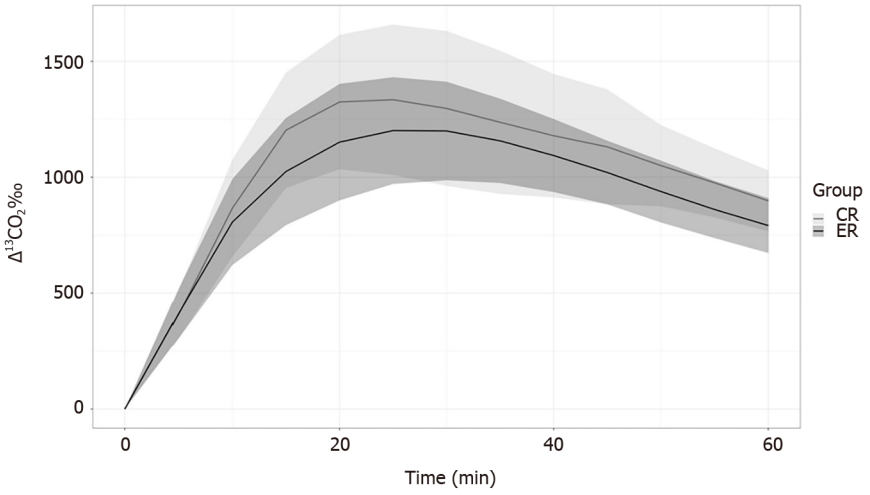

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Figure 4 Comparison of propionate breath test results between the female ethanol-fed rat and female control rat groups. Note: Tinted area around each line indicates the standard deviation range. CR: Control rat; ER: Ethanol-fed rat.

intestinal bacteria, such as *Phascolarctobacterium*, and as a trigger for changes in gut flora. Thus, we hypothesized that chronic alcohol consumption promotes propionate production both as a substrate for various fatty acids and as a trigger for changes in gut flora.

Alternatively, these observations may be due to the well-known difficulties in recapitulating the effects of chronic alcohol consumption in an animal model. We intended to establish a rat model of chronic alcoholism to evaluate the metabolic effect of ethanol consumption by oral administration of a 16% ethanol solution (corresponding to the level of alcohol commonly consumed by Japanese drinkers in the form of sake) for > 20 wk based on a previous study[16]. Therefore, we expected higher serum ALT levels in the ER group. Our contrasting result (lower serum ALT after 16% ethanol consumption) highlights the difficulty in the development of alcoholic animal models. A recent review on the utility of animal models for alcoholic liver disease mentioned that, in contrast to primates, rodent models fail to sufficiently display the full disease spectrum of alcoholic liver disease found in humans, despite many trials under various conditions[17]. The absence of craving in rats, owing to their natural aversion to ethanol[18,19], the faster ethanol catabolism in rodents than in humans[20], and differences in the innate immune systems of the species, have been proposed as the main factors contributing to the difficulty in establishing a useful rat model of human alcoholism[21]. It is therefore possible that our results also reflect failure to generate a chronic alcoholism rat model; thus, studies using primates or other small animals rather than rodents may be more appropriate. Considering the *ad libitum* diet administration, and the higher body weight in the CR group, fatty liver due to excessive dietary intake may explain the higher serum ALT levels in CRs. Because all of the rats consumed the same diet, it is possible that

consumption of 16% ethanol solution had a protective effect against liver damage. Given that propionate itself reportedly has protective effects against steatohepatitis[7], and that improvement of gut flora is an effective way to suppress liver damage[6], enhanced propionate metabolism and favorable changes in the gut flora might suppress liver damage in male ERs. As discussed earlier, the lack of acceleration in propionate metabolism in female ERs can be explained by sex-related differences in carbohydrate metabolism.

In addition to the lack of confirmation of the chronic alcoholism model, our study has other limitations. For instance, the serum methylmalonic acid (MMA) level, rather than the serum VB_{12} level, is required for the precise diagnosis of VB_{12} deficiency in humans[3]. However, we were not able to evaluate MMA levels because major domestic commercial laboratories no longer perform MMA testing of human serum or urine, and we could not find or access domestic laboratories measuring serum MMA in animal samples. Similarly, comparing the PBT results with biomarkers, such as aldehyde dehydrogenase and alcohol dehydrogenase, which sensitively and precisely reflect hepatic alcohol metabolism, may provide more information[22]. We believe that comparing levels of serum MMA and the markers evaluated using the faster PBT may provide further insight into the association between VB_{12} deficiency and alcoholism. Moreover, the present study only focused on the association between propionate metabolism and VB_{12} deficiency based on a previous study on PBT[3]. However, considering the complexity of intestinal propionate production due to the variety of propionate-producing bacteria, including *Clostridium* spp., *Veillonella* spp., *Fusobacterium* spp., *Salmonella ruminantium*, and *Propionibacterium* spp., and the complexity of substrates[23], the findings obtained herein, including the promoted propionate metabolism in male ERs and sex-related difference, may have potential clinical utility and provide a basis for future research into propionate metabolism and intestinal microbiota under various conditions. For instance, comparison of findings between faster PBT and the composition or changes in gut microbiota may provide interesting information on the association between gut microbiota and their products. Despite these limitations and lack of confirmation of VB_{12} deficiency under our experimental conditions, our study highlights the influence of ethanol and sex-related differences in propionate metabolism.

CONCLUSION

We evaluated a faster PBT in which C_{max} peaked within 30 min. This PBT could serve as a substitute for conventional PBT (which takes at least 2 h) for evaluating propionate metabolism and diagnosing VB_{12} deficiency. Although we could not evaluate the usefulness of faster PBT as a diagnostic modality for VB_{12} deficiency as initially intended because we failed to create a rat alcoholism model with VB_{12} deficiency, our study suggests that chronic consumption of 16% ethanol changed the composition of fatty acids produced by the intestinal flora, likely by changing the intestinal flora composition without causing corresponding liver injury. Considering the accumulating evidence of alteration of the gut flora as one of the mechanisms of alcoholism-related health impacts[24], our study demonstrates the potential utility of the faster PBT as a non-invasive and more convenient modality to evaluate changes in the gut flora associated with ethanol consumption and various other conditions.

ARTICLE HIGHLIGHTS

Research background

The ^{13}C-propionate breath test (PBT) has been studied as a non-invasive diagnostic modality for vitamin B_{12} (VB_{12}) deficiency by utilizing the role of VB_{12} as a coenzyme of methylmalonyl-CoA mutase in propionate metabolism. Although alcoholism has been regarded as a risk factor for deficiency, studies on propionate metabolism using the PBT in individuals with alcoholism is limited. Furthermore, conventional PBT requires up to 2 hours of breath collection time, which may undermine its clinical utility.

Research motivation

The scarcity of studies regarding the PBT in alcoholism, and the possibility of improving the clinical utility of the PBT by shortening the breath collection time, motivated us to perform this study.

Research objectives

The aim of this study was to evaluate the change in propionate metabolism due to long-term ethanol consumption in ethanol-fed rats (ERs) as an animal model of chronic alcoholism. We also aimed to evaluate the utility of a faster PBT that requires only 1 hour to collect breath.

Research methods

The ERs were 18th generation descendants of F344/DuCrj rats that had been bred by replacing standard drinking water with a 16% ethanol solution. A faster PBT was performed by injecting the ^{13}C-propionate aqueous solution from the mouth to the stomach of ERs and control rats (CRs); we collected exhaled gas in bags, and measured the $^{13}CO_2/^{12}CO_2$ isotope ratio using infrared isotope spectrometry. We measured serum VB_{12} and alanine transaminase (ALT) levels *via* chemiluminescence immunoassay and the lactate dehydrogenase-ultraviolet method, respectively. We evaluated statistical differences in mean body weight, change in $^{13}CO_2$ ($\Delta^{13}CO_2$‰), peak $\Delta^{13}CO_2$‰, and serum VB_{12} and ALT, between ERs and CRs, and males and females, respectively.

Research results

Besides male dominance of body weight ($P < 0.001$), CRs weighed significantly more than ERs ($P < 0.008$). The $\Delta^{13}CO_2$ reached a peak (C_{max}) within 30 min in both sex groups, while males had a significantly higher C_{max} and $\Delta^{13}CO_2$ at 15-45 min than females ($P < 0.05$; for all pairs). Enhanced propionate metabolism was observed in male ERs relative to male CRs, and although males had higher serum VB_{12} levels than females, no prominent differences were observed between the ER and CR groups. Male CRs had notably higher ALT levels than male ERs. These results suggest that chronic ethanol consumption may trigger fatty acid production *via* intestinal bacteria and changes in gut microbiome composition.

Research conclusions

We believe that a faster (1-h) PBT could serve as a substitute for the conventional PBT, as the $\Delta^{13}CO_2$ reached a peak (C_{max}) within 30 min in both sex groups. We failed to evaluate the usefulness of the faster PBT as a diagnostic modality for VB_{12} deficiency in the chronic alcoholism rat model; however, our study suggests that instead of inducing alcoholism, chronic consumption of 16% ethanol changed the composition of fatty acids produced by the intestinal flora.

Research perspectives

Our study demonstrates the potential utility of the faster PBT as a non-invasive and more convenient modality to evaluate changes in the gut flora associated with ethanol consumption and various other conditions *via* changes in propionate metabolism.

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FOOTNOTES

Author contributions: Sasaki Y and Urita Y coordinated the study; Sasaki Y, Kawagoe N, Imai T, and Urita Y performed the experiments; Sasaki Y and Urita Y acquired and analyzed the data; Sasaki Y and Urita Y interpreted the data; and Sasaki Y and Urita Y wrote the manuscript; All authors approved the final version of the article.

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