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Regular Article

A randomized trial to examine the impact of food on pharmacokinetics of 4-phenylbutyrate and change in amino acid availability after a single oral administration of sodium 4-phenylbutyrarte in healthy volunteers

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ABSTRACT

Urea cycle disorders (UCDs), inborn errors of hepatocyte metabolism, result in the systemic accumulation of ammonia to toxic levels. Sodium 4-phenylbutyrate (NaPB), a standard therapy for UCDs for over 20 years, generates an alternative pathway of nitrogen deposition through glutamine consumption. Administration during or immediately after a meal is the accepted use of NaPB. However, this regimen is not based on clinical evidence. Here, an open-label, single-dose, five-period crossover study was conducted in healthy adults to investigate the effect of food on the pharmacokinetics of NaPB and determine any subsequent change in amino acid availability. Twenty subjects were randomized to one of four treatment groups. Following an overnight fast, NaPB was administered orally at 4.3 g/m^2 (high dose, HD) or 1.4 g/m^2 (low dose, LD) either 30 min before or just after breakfast. At both doses, compared with post-breakfast administration, pre-breakfast administration significantly increased systemic exposure of PB and decreased plasma glutamine availability. Pre-breakfast LD administration attenuated plasma glutamine availability to the same extent as post-breakfast HD administration. Regardless of the regimen, plasma levels of branched-chain amino acids (BCAA) were decreased below baseline in a dose-dependent manner. In conclusion, preprandial oral administration of NaPB maximized systemic exposure of the drug and thereby its potency to consume plasma glutamine. This finding may improve poor medication compliance because of the issues with odor, taste, and pill burden of NaPB and reduce the risk of BCAA deficiency in NaPB therapy.
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1. Introduction

The urea cycle, a biochemical pathway in mammals, converts highly toxic ammonia to urea and is the predominant mechanism of disposal of waste nitrogen arising from the catabolism of dietary and endogenous proteins. Urea cycle disorders (UCDs), a group of inborn errors of hepatocyte metabolism, are caused by a deficiency of enzymes or transporters involved in ureagenesis and result in systemic accumulation of ammonia at toxic levels [1]. Treatment of UCDs must be prioritized to prevent hyperammonemia because this can cause neurocognitive deficits that have a serious impact on a patient's intellectual ability and lead to coma and even death [2]. Sodium 4-phenylbutyrate (NaPB) is approved for chronic adjunctive treatment of UCDs. It is converted to 4-phenylacetate (PA) by β-oxidation and then to 4-phenylacetylglutamine (PAG) by conjugation of PA with glutamine, the major reservoir of nitrogen [3]. Therefore, NaPB generates an alternative pathway of nitrogen deposition that replaces the urea cycle through urinary excretion of PAG and consequently reduces the systemic ammonia level in UCDs [4]. NaPB therapy has been the standard treatment for long-term management of UCD for over 20 years. The approved regimen is that the drug is taken with, or immediately after, a meal [5]. However, there is no clinical evidence from which this regimen was determined.

Abbreviations: AAs, amino acids; AUC_{0-10}, area under the plasma concentration–time curve from time 0 to 10 h; BCAA, branched-chain amino acids; CI, confidence interval; C_{max}, the maximum plasma concentration; HD, high dose; $iAUC_{0-5}$, incremental area under the curve from time 0 to 5 h after breakfast; K_{el}, elimination rate constant; LD, low dose; NaPB, sodium 4-phenylbutyrate; PA, 4-phenylacetate; PAG, 4-phenylacetylglutamine; PB, 4-phenylbutyrate; PFIC, progressive familial intrahepatic cholestasis; PK, pharmacokinetics; SD, standard deviation; $t_{1/2}$, elimination half-life; T_{max}, time to reach C_{max}; UCDs, urea cycle disorders.

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We have identified another pharmacological effect of NaPB. We showed that it increases the hepatocanalicular expression of a bile salt export pump, an ATP-binding cassette transporter that mediates biliary excretion of bile acids from hepatocytes and provides the osmotic driving force for bile flow formation [6,7]. To examine the efficacy and safety of NaPB in intrahepatic cholestasis, we performed clinical studies in progressive familial intrahepatic cholestasis (PFIC) [8–10], a rare inherited autosomal recessive liver disease [11,12]. We showed that preprandial oral administration rather than prandial or postprandial administration approved for treating UCD could maximize the potency of NaPB therapy in PFIC because food intake before the administration of NaPB markedly reduced the systemic exposure to PB and could diminish its therapeutic efficacy in PFIC patients [9]. Importantly, no severe adverse events attributable to the administration of NaPB were observed during preprandial oral NaPB therapy.

Patients with UCD have difficulty taking NaPB because of its odor, taste, high sodium content, high pill burden, and high economic cost, which results in poor medication compliance and hence worsens their therapeutic outcome [13]. We reasoned that food interactions with the pharmacokinetics (PK) and pharmacodynamics (PD) of NaPB might occur in patients with diseases other than intrahepatic cholestasis and may confer higher clinically effective doses of NaPB in UCD patients. To gain an insight into the optimal regimen for NaPB treatment in UCD patients, we performed a randomized, single-center, open-label, five-period, four-sequence, crossover study of NaPB in 20 healthy volunteers. We evaluated the effect of meal timing on the PK of NaPB and the change in amino acid (AA) availability induced by NaPB. Plasma levels of glutamine were measured to determine the efficacy of NaPB on nitrogen excretion. Because of the increased risk of branched-chain amino acid (BCAA) deficiency in UCD patients undergoing NaPB treatment [14], essential AAs including BCAA in plasma were also monitored.

2. Materials and methods

2.1. Ethics statement

This study was approved by the institutional review boards at the University of Tokyo and Juntendo University, and was performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (as revised in Edinburgh 2000). Informed consent was obtained from all subjects before enrollment in the study. The study was registered in the UMIN Clinical Trials Registry at http://www.umin.ac.jp/ctr/index.htm (UMIN000027674).

2.2. Subjects

The participants were 20 healthy Japanese volunteers and comprised 7 males and 13 females. The mean ± standard deviation (SD) values of their age, height, body weight, and body mass indices were 29.3 ± 5.7 years (range, 20–40 years), 163.7 ± 9.0 cm (range, 149.0–180.5 cm), 57.7 ± 10.3 kg (range, 44.1–77.9 kg), and 21.5 ± 3.4 (range, 17.6–26.1) (Table 1). Each subject was physically normal, over 20 years old, and had no antecedent history of significant medical illness or hypersensitivity to any drugs. Their health status was judged by the clinical investigator to be normal based on a full physical examination with a screening of blood chemistry and complete blood count before the study (Supplementary Table 1). None of the participants had taken any drugs or beverages containing grapefruit, orange, or apple juice and they were prohibited from smoking and consuming alcohol for at least 2 months before, and during, the study.

2.3. Study design

To examine the effect of meals on the PK of NaPB and its effects on the availability of AA in healthy subjects, a randomized, single-center, open-label, five-period, four-sequence, crossover study of NaPB was conducted in Juntendo University Hospital. Twenty eligible subjects as described above were enrolled by the physicians in this study between November 2017 and November 2018. Each participant was randomly assigned to one of four sequences in a 1:1:1:1 ratio (Fig. 1). The randomization list was generated by permuted block randomization, with a block size of four, and prepared by an individual who was not involved in patient care. Each sequence consisted of five periods including the four treatment regimens in different orders and non-treatment period. The four treatment regimens were: pre-breakfast oral administration of NaPB (Buphenyl; OrphanPacific, Tokyo, Japan) at 4.3 g/m^2 (high dose, HD); post-breakfast oral administration of NaPB at 4.3 g/m^2 (HD); pre-breakfast oral administration of NaPB at 1.4 g/m^2 (low dose, LD); and post-breakfast oral administration of NaPB at 1.4 g/m^2 (LD). HD is the upper limit of approved prescribing when used three times a day, whereas LD is a common dose in current clinical usage [5].

The subjects were given NaPB orally, either HD or LD 30 min before breakfast or just (<10 min) after breakfast following an overnight fast. Participants were each treated four times receiving NaPB both HD and LD pre- and post-breakfast in the same combinations. The period without NaPB therapy were performed following an overnight fast. Each period was separated by a washout period of more than 24 h. The washout period was based on the previous study, which reported that the majority of PB and its metabolites disappear from systemic exposure and are excreted into the urine within 24 h of oral administration of NaPB [5,15]. A standard meal was served at breakfast, and at 4 and 10 h post-treatment. All subjects were given the same breakfast (707 kcal) consisted of 29.6 g protein, 27.0 g lipid, and 86.2 g sugar and prohibited from ingesting any food for at least 4 h after NaPB administration.

Blood samples were collected through a catheter placed in a forearm vein into an EDTA-2Na$^+$-pretreated tube before dosing and at 0, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480, and 600 min after drug administration. Blood samples were placed at 4 °C immediately after collection and centrifuged for 15 min at 1700 ×g to prepare plasma. The prepared specimens were stored at −80 °C until measurement.

2.4. Quantification of PB concentrations in human plasma [9]

After the addition of 4-phenylbutyric acid-d_{11} (Toronto Research Chemicals Inc., Ontario, Canada) as the internal standard, the PB and internal standard present in the human plasma were deproteinized with acetonitrile. The supernatant was mixed with 0.01% formic acid and subjected to quantitation on an AB SCIEX QTRAP 5500 mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a Prominence high-performance liquid chromatography system (Shimadzu Corporation, Kyoto, Japan), operated in the negative electrospray ionization mode. Chromatographic separation was achieved at 40 °C on a Gemini

Table 1
Demographic data in healthy subjects.

	Male (N = 7)	Female (N = 13)
Age (years) at screening		
Mean (SD)	31.9 (4.9)	27.9 (5.7)
Median	31	29
Minimum/maximum	27/40	20/35
BMI (kg/m^2)		
Mean (SD)	21.6 (2.3)	21.4 (3.9)
Median	21.3	19.8
Minimum/maximum	19.1/24.1	17.6/31.9
Height (cm)		
Mean (SD)	171.5 (7.9)	159.5 (6.6)
Median	173.5	161.5
Minimum/maximum	156.5/180.5	149.0/168.0
Body weight (kg)		
Mean (SD)	63.9 (10.4)	54.4 (8.9)
Median	66.0	51.5
Minimum/maximum	46.7/77.9	44.1/72.7

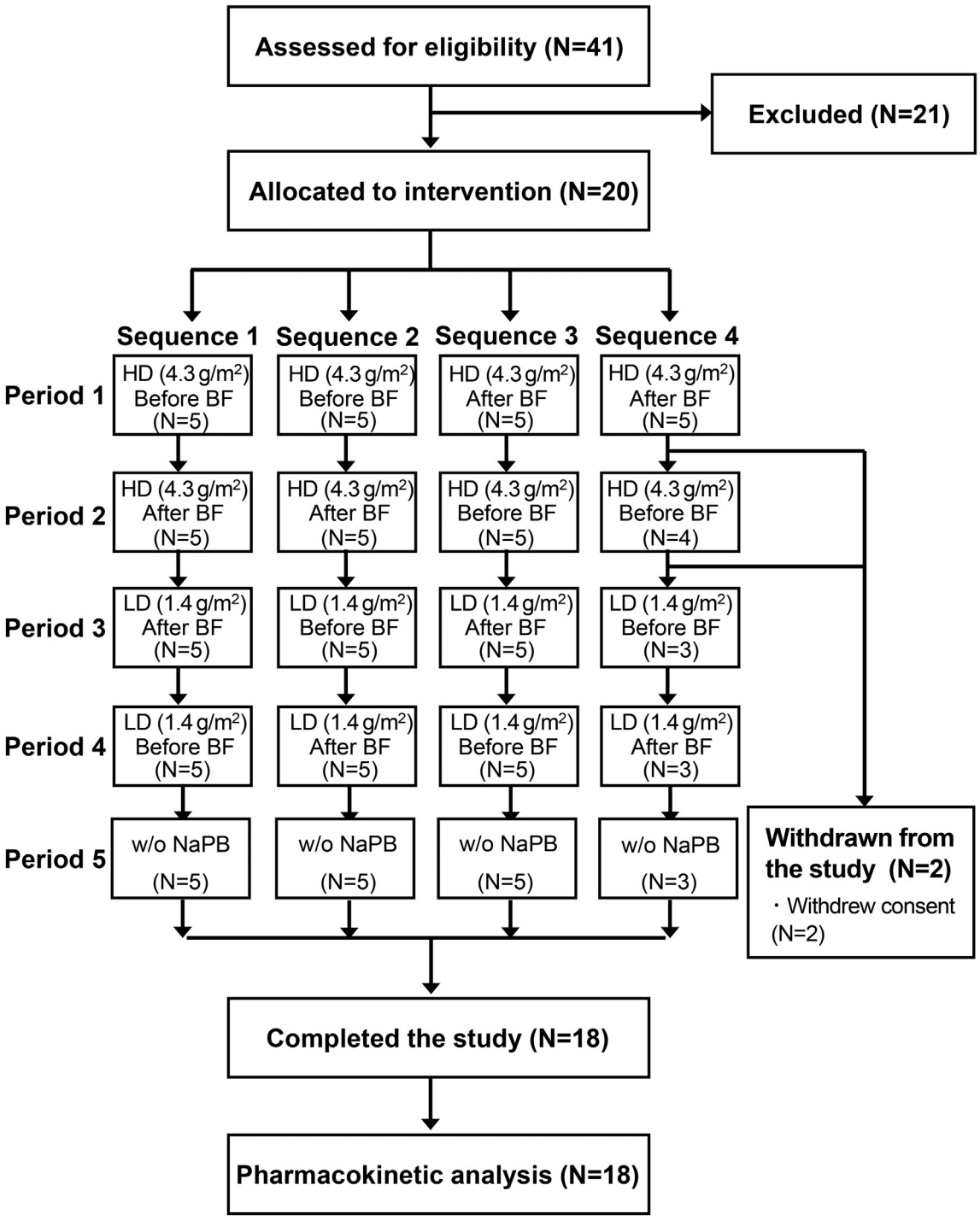

Fig. 1. Flow diagram of this study. BF, breakfast; HD, high dose; LD, low dose; w/o NaPB, without NaPB treatment.

C-18 column (150 × 2 mm, 3 μm; Phenomenex, Torrance, CA) in binary gradient mode at a flow rate of 0.4 mL/min. The mobile phase comprised acetonitrile containing 0.01% formic acid. The acetonitrile concentration was initially 10%; it was then linearly increased to 80% over 1 min and maintained for a further 1.1 min. Finally, the column was re-equilibrated with acetonitrile at a concentration of 10% for 1.8 min. The mass transition was from *m/z* 162.9 to 90.9 for PB and from m/z 174.1 to 98.0 for the internal standard. The analytical method was validated in terms of selectivity and linearity (0.5–200 μg/mL in human plasma, correlation coefficient (r) ≥ 0.99).

2.5. Quantification of AA concentrations in human plasma

Blood levels of 9 AAs (glutamine, leucine, isoleucine, valine, threonine, methionine, histidine, phenylalanine, and lysine) were measured. Plasma was mixed with an equal volume of 140 μM sulfosalicylic acid and 300 μM 5-aminovaleric acid as the internal standard. The mixture was centrifuged at 20,000 ×*g* for 15 min at 4 °C. The supernatant was loaded onto an ultrafiltration filter (Ultrafree-MC, 0.45 μm, hydrophilic PTFE; Millipore, Bedford, MA) and centrifuged at 2500 ×*g* for 5 min at 4 °C. The lower layers were collected and subjected to AA measurement.

The amount of each AA was determined using a high-speed AA analyzer L-8900FF (Hitachi High-Technologies Corporation, Tokyo, Japan). AAs were separated using ion-exchange chromatography and detected spectrophotometrically after post-column reaction with ninhydrin.

2.6. Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) of PB were determined directly from the observed data. The area under the plasma concentration–time curve from time 0 to 10 h after the administration of NaPB (AUC_{0-10}) of PB was calculated using the linear trapezoidal rule. The elimination rate constant (K_{el}) of PB was estimated using least-squares regression analysis from the terminal post-distribution phase of the concentration–time curve. The elimination half-life ($t_{1/2}$) of PB was calculated as 0.693 divided by K_{el}.

The incremental area under the curve from time 0 to 5 h after breakfast ($iAUC_{0-5}$) for each AA was defined as the change in plasma AA values from baseline and calculated for each subject using the linear trapezoidal rule.

2.7. Statistical analysis

Based on our previous PK study of NaPB in PFIC patients [9], we calculated that a sample size of 20 participants, with 18 evaluable participants, would provide the trial 80% power to detect a significant difference in AUC_{0-10} and C_{max} of PB and $iAUC_{0-5}$ of glutamine between pre-breakfast and post-breakfast regimens at an alpha level of 0.05 (two-sided) based on a two-sample *t*-test.

Data are shown as means ± SD, unless otherwise indicated. Statistical analyses were performed using Prism software (v. 6; GraphPad Software, La Jolla, CA) and Pingouin, a free statistics library of Python (v. 3.6). The differences between the treatment regimens in C_{max}, AUC_{0-10}, T_{max}, and $t_{1/2}$ of PB, and $iAUC_{0-5}$ of AA were evaluated with one-way Welch analysis of variance and subsequent Games–Howell test considering unequal variances [16,17].

3. Results

Twenty subjects (7 males and 13 females) were enrolled, and 18 subjects (7 males and 11 females) completed the defined protocol (Fig. 1). The demographic characteristics of the subjects are outlined in Table 1. Of the two subjects withdrawn from the study, one withdrew consent after period 1 and the other after period 2 because of personal reasons. Their data were excluded from the analysis of PK of PB (Fig. 2) and plasma AAs (Fig. 3). No clinically undesirable signs or symptoms attributable to the administration of NaPB were detected during the study.

3.1. Pharmacokinetics of PB

The mean plasma concentration–time curves of PB after a single oral dose of NaPB for each regimen are shown in Fig. 2. To evaluate the food effect on PK of PB, the PK parameters were calculated (Table 2 and Supplementary Table 2). At both HD and LD, although more prominent at LD, post-breakfast administration markedly reduced the plasma concentration of PB. The C_{max} and AUC_{0-10} of PB for post-breakfast administration were both lower than those for pre-breakfast administration by 1.37 times (95% confidence interval (CI), 1.25–1.49; $P = 0.001$) and 1.21 times (95% CI, 1.09–1.32; $P = 0.13$) at HD, and 2.33 times (95% CI, 1.91–2.53; $P = 0.001$) and 1.59 times (95% CI, 1.43–1.75; $P = 0.003$) at LD, respectively. At both doses, the T_{max} of PB after post-breakfast administration occurred later than after pre-breakfast administration. At LD, but not HD, the K_{el} of PB was markedly decreased by breakfast intake before administration of NaPB. Consequently, the $t_{1/2}$ of PB for post-breakfast LD administration of NaPB was 1.81 times (95% CI, 1.59–2.12; $P = 0.003$) longer than for its pre-breakfast administration.

3.2. Effect of NaPB on plasma AAs

The mean values of changes in plasma glutamine, BCAA, and the other essential AAs at each time point after breakfast relative to each regimen of a single oral NaPB dose are shown in Fig. 3A–C. To evaluate the effect of NaPB on AA intake from breakfast, an iAUC value during the period after breakfast to lunch ($iAUC_{0-5}$) for glutamine, BCAA, and the other essential AAs was calculated (Fig. 3D–F and Supplementary Table 3).

NaPB attenuated $iAUC_{0-5}$ of glutamine at both HD and LD, and its effect was more prominently observed in the pre-breakfast than post-breakfast regimens (Fig. 3D). The difference in $iAUC_{0-5}$ of glutamine between pre-breakfast administration and no NaPB treatment was lower than that between post-breakfast administration and no NaPB treatment: pre-breakfast (95% CI, −50,460 to −34,700) vs post-breakfast (95% CI, −34,020 to −19,770) at HD ($P = 0.001$), and pre-breakfast (95% CI, −24,840 to −12,840) vs post-breakfast (95% CI, −12,490 to −18,410) at LD ($P = 0.001$). No statistically significant differences were found in $iAUC_{0-5}$ of glutamine between post-breakfast HD administration and pre-breakfast LD administration. NaPB reduced $iAUC_{0-5}$ of

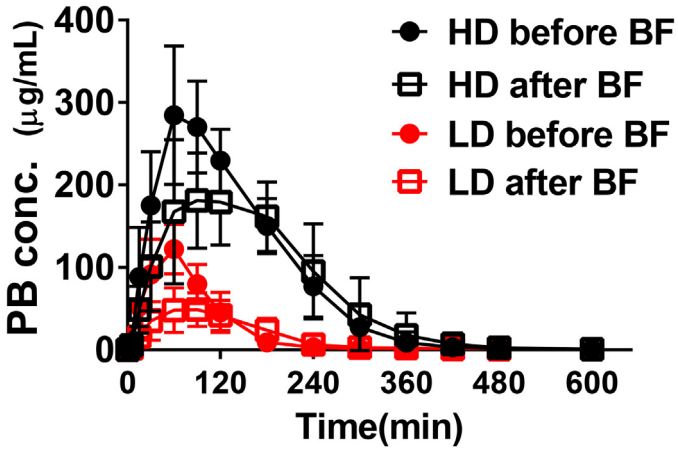

Fig. 2. Effect of food on the systemic exposure and PK parameters of PB after oral administration of NaPB in healthy subjects. NaPB (4.3 g/m^2 (HD), 1.4 g/m^2 (LD)) was given orally to healthy subjects either 30 min before or just (<10 min) after breakfast following an overnight fast. Systemic exposure. Plasma concentrations of PB were determined at the times shown. Data are shown as means ± SD ($N = 18$) of the plasma concentrations. BF, breakfast; HD, high dose; LD, low dose.

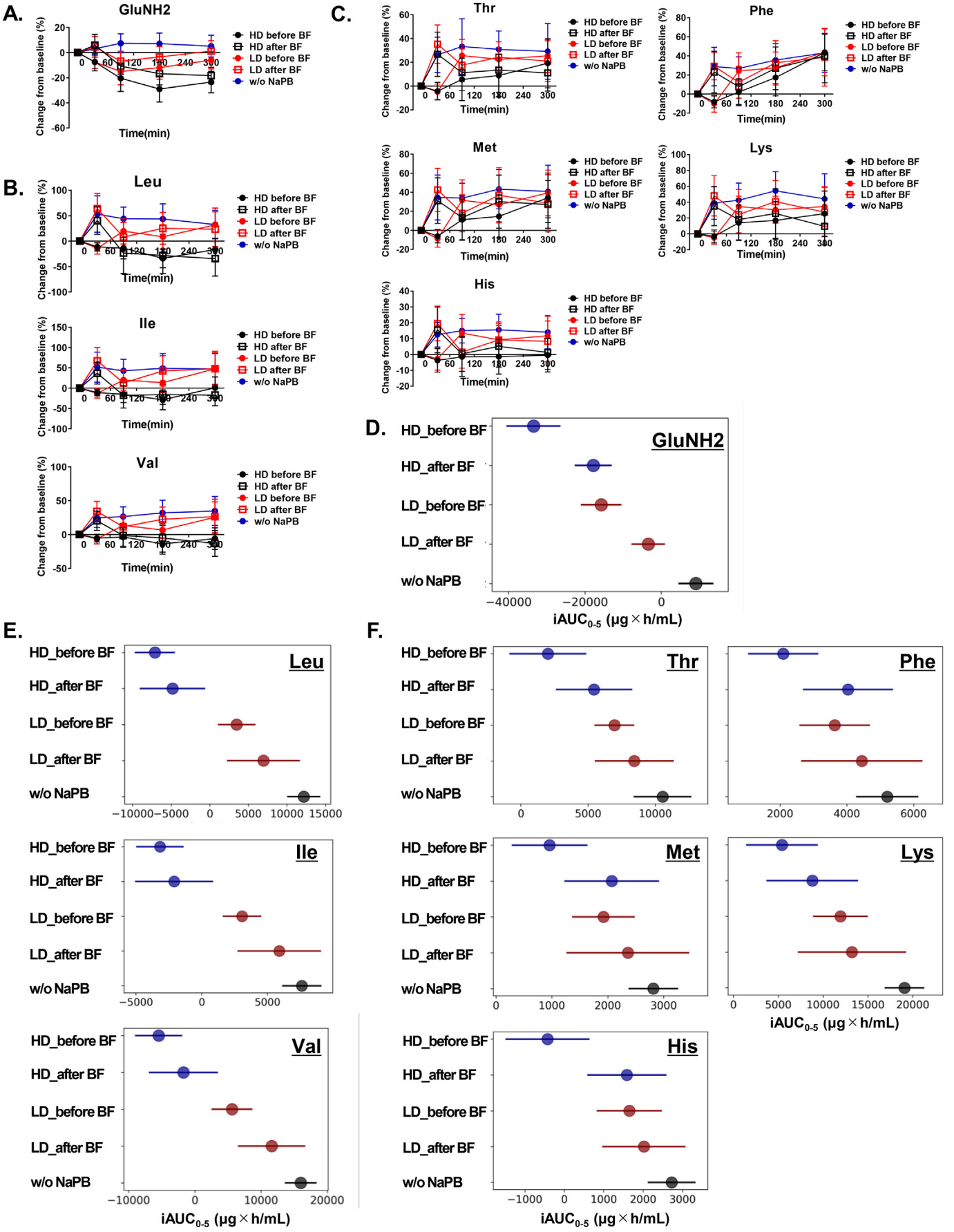

Fig. 3. Effect of NaPB on AA availability after breakfast in healthy subjects. NaPB (4.3 g/m^2 (HD), 1.4 g/m^2 (LD)) was given orally to healthy subjects either 30 min before or just (<10 min) after breakfast following an overnight fast. (A–C) Change rate from the baseline of glutamine (A), BCAAs (B), and the other essential AAs (C) after breakfast. Plasma concentrations of each AA were determined at the times shown. Change rate from the baseline (pre-breakfast concentration) is calculated for each AA and is shown as means ± SD (N = 18). (D—F) Availability of glutamine (D), BCAAs (E), and the other essential AAs (F) after breakfast. $iAUC_{0-5}$ of AAs was calculated as described in Materials and Methods. Data are presented as means (circles) and 95% CI (horizontal lines) (N = 18). BF, breakfast; GluNH2, glutamine; HD, high dose; His, histidine; Ile, isoleucine; LD, low dose; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine; w/o NaPB, without NaPB treatment.

Table 2
Plasma pharmacokinetc parameters of PB in healthy subjects administered with NaPB.

	HD		LD	
	Before BF	After BF	Before BF	After BF
C_{max} (μg/mL)	305.5 [277.7–330.3]	228.4 [204.1–252.8]	129.9 [117.3–142.5]	62.7 [53.7–71.6]
T_{max} (h)	1.0 [1.0–2.0]	1.8 [1.0–4.0]	1.0 [0.5–1.0]	1.3 [0.5–3.0]
AUC_{0-10} (μg × h/mL)	804.9 [717.9–891.9]	681.0 [618.4–743.5]	197.5 [170.9–224.2]	131.9 [108.9–155.0]
$t_{1/2}$ (h)	0.60 [0.49–0.71]	0.75 [0.62–0.87]	0.49 [0.35–0.63]	0.93 [0.74–1.12]

Data are shown as mean (95% confidence interval); T_{max} data are shown as median (range). BF, breakfast.

BCAA in a dose-dependent manner (Fig. 3E). The food effect on $iAUC_{0-5}$ was not evident for BCAA at both HD and LD. Pre-breakfast HD administration, but not the other regimens, reduced $iAUC_{0-5}$ of the other essential AAs (Fig. 3F).

4. Discussion

This study examined the impact of food on PK of PB and change in AA availability after a single oral administration of NaPB in healthy subjects at two doses, 4.3 g/m^2 (HD), an upper limit of approved labeling when used three times a day, and 1.4 g/m^2 (LD), a common dose in clinical usage [5]. For both doses, breakfast intake before the administration of NaPB markedly reduced the systemic exposure to PB and thereby its potency to consume plasma glutamine (Figs. 2 and 3D). The food effect was not reflected in a change in availability for BCAA after NaPB administration (Fig. 3E).

NaPB has been established as standard therapy for long-term management of UCD for at least two decades [18]. However, the optimal regimen for NaPB therapy has never been fully examined. Consistent with our previous PK study of a single oral dose of NaPB in seven pediatric patients with intrahepatic cholestasis [9], here, we showed that pre-breakfast administration, rather than the usual post-breakfast administration was preferable to increase systemic exposure of PB in healthy subjects (Fig. 2). After intestinal absorption, PB is taken up by hepatocytes and predominantly converted to PA through β-oxidation. PA forms PAG through conjugation with glutamine and is then excreted into the urine [15]. The longer time until T_{max} observed with post-breakfast administration of NaPB (Table 2) suggests that the reduction in PB systemic exposure as a result of food intake is attributable to inhibition of the intestinal absorption of PB as well as the ionization of PB by changes of the gastric pH and/or protein binding.

Plasma glutamine levels are predictors of hyperammonemia in UCDs [19]. In both HD and LD of NaPB, food intake lessened the potency of the drug in its ability to decrease plasma glutamine levels (Fig. 3D). Pre-breakfast LD administration of NaPB attenuated $iAUC_{0-5}$ of glutamine to the same extent as post-breakfast HD administration, suggesting that the change in regimen to preprandial administration maximizes the potency of NaPB to clear plasma glutamine and decreases its clinically effective dosage.

The effect of food on NaPB was more prominently observed in its potency to consume plasma glutamine (Fig. 3D) than in C_{max} and AUC_{0-10} of PB (Table 2). Considering that the food effect was observed in the elimination process of PB from blood (Table 2), food ingestion could affect the hepatic uptake of PB and/or PB β-oxidation to form PA in hepatocytes, leading to less hepatic exposure to PA than that predicted by systemic exposure of PB. Alternatively, the delayed elimination phase of PB could be explained by flip-flop pharmacokinetics due to the short half-life of PB. Understanding the molecular mechanisms responsible for the food effect on PK of PB will make it possible to gain greater insight into the PK/PD relationship of NaPB and prescribe NaPB based on genetic variability, drug–drug interactions, and drug–nutrient–food interactions.

NaPB decreased BCAA levels in UCD patients [1,20]. This could be because of PB-mediated activation of branched-chain α-keto acid dehydrogenase, a multienzyme complex of BCAA catabolism that catalyzes decarboxylation of the branched-chain α-keto acid to the corresponding branched-chain acyl-CoA esters [21,22]. BCAA deficiency has been reported to retard growth, promote protein wasting, and adversely affect brain function [22–24], and is thought to be the cause of several adverse events in UCD patients undergoing NaPB therapy. We observed a marked and slight reduction in $iAUC_{0-5}$ of BCAA at HD and LD of NaPB, respectively, despite sufficient total protein intake at breakfast (Fig. 3E). In neither dose was the food effect on NaPB treatment evident for $iAUC_{0-5}$ of BCAA. This finding provides useful information for the development of an evidence base for the treatment of UCDs. It also informs the treatment of maple syrup urine disease, a classical inborn error of AA metabolism that causes an accumulation of BCAA in the body and manifests as potentially lethal episodes of intoxication [21]. Preprandial dosing makes it possible to decrease the effective clinical dosage of NaPB and thereby prevent BCAA depletion in UCDs, whereas HD, an upper limit of approved dose for UCDs, should be prescribed to solve aberrant accumulation of BCAA in maple syrup urine disease.

A limitation of this study was that it was conducted on healthy subjects. Although a major goal of NaPB therapy is the management of UCDs by preventing hyperammonemia, plasma ammonia levels in healthy subjects are well controlled and any decrease caused by NaPB treatment is difficult to detect. This study assessed plasma glutamine levels, which have been shown to correlate better with clinical symptoms in UCDs [19]. Thus, effects beyond those in the population studied, especially concerning disease state, cannot be adequately inferred. However, it is conceivable that the findings on the PK of PB in this study apply to other diseases, including UCDs, because the food impact on PK of PB was similar to that seen in our previous study in pediatric patients with intrahepatic cholestasis, who varied in age and disease severity [9].

In conclusion, our present study showed that food intake before the administration of NaPB markedly reduced the systemic exposure to PB and its therapeutic efficacy to consume glutamine. Therefore, preprandial instead of the approved prandial or postprandial oral administration of NaPB may be preferable to maximize its therapeutic potency, allow a decrease in the clinically effective dose, and prevent BCAA depletion in UCD patients. This finding makes it possible to ameliorate the difficulties associated with NaPB therapy because of its odor, taste, high pill burden, and high economic cost. These features induce poor medication compliance and worsen the therapeutic outcome [13], and their control may improve the cost-effective management of UCD patients. Future studies in UCD patients should assess preprandial treatment with NaPB with careful monitoring of adverse events such as PA-induced neurotoxicity [25] and hepatotoxicity [26].

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Author contributions

S.O. designed the pharmacokinetic study, measured plasma concentration of PB, and wrote the manuscript. S.N. designed and performed the pharmacokinetic study. T.M. performed statistical analysis and wrote the manuscript. S.H., Y.S., and K.M. performed the pharmacokinetic study. A.M. analyzed the data. Y.H. and Y.M. measured plasma

concentration of amino acids. H.K. and T.S. revised the manuscript for intellectual content. M.S. designed the pharmacokinetic study and revised the manuscript for intellectual content. H.H. conceived, designed, supervised the study, analyzed the data, and wrote the manuscript. All authors approved the manuscript before submission.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2021.02.002.

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