

# Changes in plasma-concentration ratios of branched-chain amino acids in acute and convalescent phases of bacterial pneumonia

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Branched-chain amino acids (BCAAs) have different immunity-related functions. Thus, BCAAs require evaluation in terms of their plasma concentration ratio. Eighty healthy participants and 57 patients with community-acquired pneumonia were enrolled. Samples from the healthy participants were collected after 12-h fasting; samples from the community-acquired pneumonia group were collected 2–3 h after lunch, during the acute (day 0) and convalescent (day 7) phases. The coefficient “a” of the regression line ( $Y = aX + b$ ) of each BCAA plasma concentration was calculated from healthy participants and fixed, and each intercept “b” was calculated from the plasma concentration of each BCAA pair. Isoleucine levels increased; no significant changes in leucine concentrations were observed between healthy participants and pneumonia patients on days 0 and 7. In female participants in the pneumonia group, valine concentrations decreased on day 0. The isoleucine concentration was relatively higher than the leucine concentration on day 7 when evaluated with “b”. Changes in “b” on days 0 and 7 differed between men and women. There were sex-related differences in the plasma concentration ratios of BCAAs evaluated by “b”, which indicates a possible sex-related difference in the metabolic response to bacterial infection.

**Key Words:** bacterial pneumonia, branched-chain amino acid, plasma concentration, cross-correlation, analysis of intercept

Since the late 1990s, the possibility of improving immune function through supplementation of specific amino acids, such as glutamine and arginine, and nutrients, such as  $\omega$ -3 fatty acids, has drawn attention under the niche concept of immunonutrition.<sup>(1,2)</sup> Three branched-chain amino acids (BCAAs)—isoleucine (Ile), leucine (Leu), and valine (Val)—have anti-inflammatory properties against lipopolysaccharides (LPS)<sup>(3)</sup> and are essential amino acids that play key roles in biological functions such as protein synthesis and immune cell activity.<sup>(4)</sup> BCAAs account for 35% of the essential amino acids present in muscles,<sup>(5)</sup> and muscles are the largest storage organ for BCAAs in the body.<sup>(6)</sup> In a bacterial infection, various cytokines, such as interleukin (IL)-6, are involved in the immune response,<sup>(7)</sup> and these infections affect the plasma concentration of BCAA ([BCAA]) because IL-6 and endotoxins reduce the protein turnover in human muscles.<sup>(8,9)</sup> BCAA uptake in the mitochondria of brown adipose cells controls the homeostasis of body temperature and influences [BCAA] during inflammatory states.<sup>(10)</sup> It is speculated that BCAA metabolism in brown adipocytes changes during infection and affects [BCAA], especially in patients with bacterial pneumonia. As amino acids are essential nutrients for the host and the pathogen in infectious diseases,<sup>(11)</sup> the metabolic crosstalk between the host and the pathogen regarding amino

acids is attracting attention.<sup>(12,13)</sup> Regarding BCAA interrelationships, Ile and Val suppress Leu metabolism in human peripheral lymphocytes,<sup>(14)</sup> and each BCAA has a different effect on immune function.<sup>(15)</sup> Therefore, the ratios of specific BCAA are considered an important factor in the evaluation of BCAAs from the immunonutrition perspective. Each [BCAA] demonstrated a strong correlation with the other [BCAAs],<sup>(16–18)</sup> and these correlations are strongly maintained even in chronic renal failure, which induces severe amino acid-related metabolic disorders.<sup>(19)</sup> Moreover, as the correlations form regression lines that do not pass through the origin, the ratios of the BCAA concentrations will differ depending on their corresponding concentration. Thus, a method of evaluation that uses the “b” value, which is calculated from the measured values of each pair of [BCAAs], and fixes the coefficient “a” of the slope of the regression line ( $Y = aX + b$ ), was proposed.<sup>(19)</sup> There are a few detailed reports of amino-acid changes during bacterial pneumonia in humans; therefore, changes in plasma amino acid levels in bacterial pneumonia cases during the acute and convalescent phases were previously evaluated.<sup>(20)</sup> In this study, the ratios of BCAAs were analyzed using the proposed evaluation method, starting from the acute phase and extending until the convalescent phase of bacterial pneumonia. Additionally, the values and the ratios of [BCAA] were compared to assess for metabolic changes in BCAA during bacterial pneumonia.

## Methods

Healthy older adult participants (control group: C) underwent health examination at Sanyudo Hospital between May 2018 and December 2018. However, data pertaining to participants who were being treated with medications or had diabetes [glycated hemoglobin (HbA1c) >6.1% or taking medications for diabetes treatment], chronic renal failure [estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m<sup>2</sup>], or malignant disease were excluded from the analysis. Moreover, individuals whose [BCAA] values deviated from the reported Japanese standard values<sup>(21)</sup> were excluded from the analysis. The reference intervals for [BCAA]s are as follows: 36.4–85.0 nmol/ml for [Ile], 76.7–159.5 nmol/ml for [Leu], and 143.0–287 nmol/ml for [Val]. The bacterial pneumonia group included patients with community-acquired pneumonia (CAP) who were admitted to our hospital between March 2016 and December 2019. However, only patients who had not received antibiotics prior to hospitalization and whose fever and cough symptoms had started within the last 3 days before hospitalization were enrolled in this study.

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**Table 1.** Age of participants

	Control group		CAP group		p value
	n	Age (mean ± SD)	n	Age (mean ± SD)	
Male	44	70.5 ± 7.36	31	77.3 ± 13.7	<0.001
Female	36	70.4 ± 7.26	24	82.2 ± 12.0	<0.002

AP, community acquired pneumonia.

**Table 2.** Results of laboratory examination of CAP group

Parameters	Day 0	Day 7	p value
	n = 31	n = 25	
Male	Mean ± SD	Mean ± SD	
White blood cell (μl)	11,613 ± 4,223	6,911 ± 1,927	<0.001
C-reactive protein (mg/dl)	13.40 ± 8.22	2.04 ± 3.10	<0.001
Blood urea nitrogen (mg/dl)	19.1 ± 6.1	NA	NA
Female	n = 24	n = 16	
	Mean ± SD	Mean ± SD	p value
White blood cell (μl)	11,374 ± 3,928	6,201 ± 1,459	<0.001
C-reactive protein (mg/dl)	12.56 ± 7.78	1.81 ± 1.61	<0.001
Blood urea nitrogen (mg/dl)	19.5 ± 12.4	NA	NA

CAP, community acquired pneumonia.

CAP was diagnosed according to the diagnostic criteria specified in the guidelines of the Japanese Respiratory Society.<sup>(22)</sup> The characteristics of the participants in the CAP group overlapped with those of participants of a previous study.<sup>(6)</sup> Venous blood samples from the C group were collected between 8 and 9 o'clock in the morning after the participants had fasted from 9 o'clock the previous night. In the bacterial pneumonia group, 12-h fasting was not possible because treatment was prioritized, and the samples were collected 2–3 h after lunch. Samples collected within 24 h after hospitalization were designated as specimens collected on day 0, and samples were collected again 6 to 8 days into hospitalization (day 7) for some patients. The dietary BCAA content of the CAP group was approximately 2,800, 5,100, and 3,400 mg of Ile, Leu, and Val, respectively, per day. For lunch, the dietary Ile, Leu, and Val intake was approximately 1,200, 2,200, and 1,400 mg, respectively.

Immediately after collection in a collection tube containing EDTA-2Na, the venous blood sample was cooled with ice. Subsequently, the plasma was centrifuged and cryopreserved at -40°C. The [BCAAs] were analyzed by SRL, Inc. using liquid chromatography-mass spectrometry. The [BCAA] ratios were evaluated using a previously described method<sup>(19)</sup> as follows: 1) The coefficient “a” of the slope of the regression line ( $Y = aX + b$ ) in the cross-correlation between each [BCAA] was obtained from the healthy participant group. 2) Next, “a” was fixed, and each measured [BCAA] was substituted into the (X, Y) of the regression line equation ( $Y = aX + b$ ) to calculate the “b” value. 3) Finally, the calculated “b” values of the different study groups were compared.

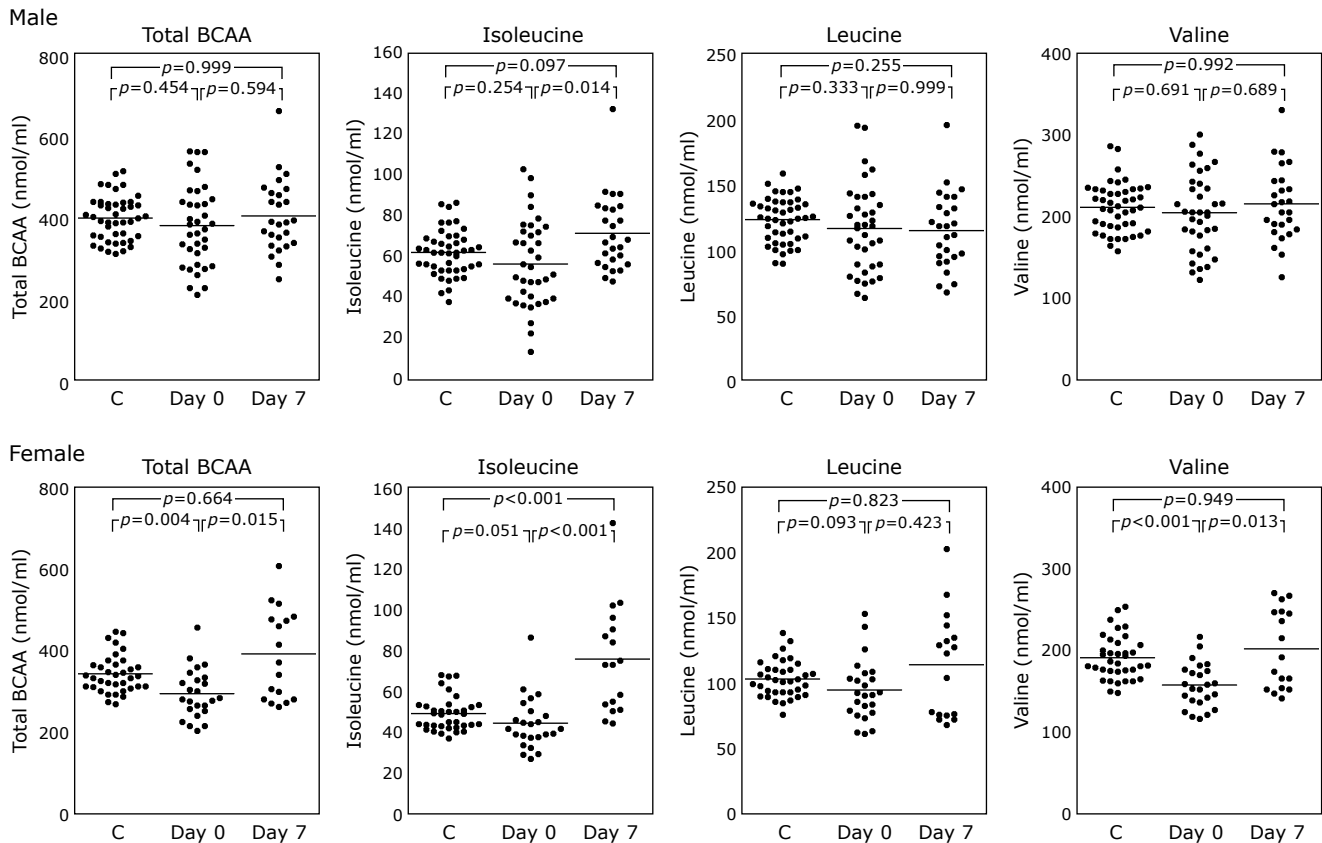
**Statistical analysis.** The Mann–Whitney *U* test was used to compare data between the groups of male and female participants. A nonparametric multiple comparison test (Steel–Dwass method) was used to compare the three study groups, which was conducted on day 0 for the C and CAP groups and on day 7 for the CAP group. The correlation between each BCAA was determined using the least-squares method.

**Ethical considerations.** This study was approved by the Sanyudo Hospital Ethics Committee at the 57th, 59th, 63rd, and 65th ethics committee meetings. Written informed consent for study participation was obtained from all participants in accordance with the Declaration of Helsinki. These processes were performed in accordance with the Ministry of Health, Labour and Welfare’s “Ethical Guidelines for Medical and Health Research Involving Human Subjects”.<sup>(23)</sup>

## Results

This study included 80 participants (44 males and 36 females) in the C group and 57 (33 males and 24 females) in the CAP group (Table 1). Both male and female participants were older in the CAP group than in the C group. Table 2 shows the white blood cell count (WBC) and levels of C-reactive protein (CRP) and blood urea nitrogen (BUN) on days 0 and 7 in the CAP group. Antibiotic administration was completed by day 7, and no recurrence of pneumonia was observed. Both WBC and CRP levels were significantly lower on day 7 than on day 0.

In Fig. 1, the [BCAA] of the C and CAP groups on days 0 and 7 were compared separately between male and female participants. The total [BCAAs] decreased on day 0 for females; however, there was no difference among the three groups for males. Compared to the C group, the plasma concentration of Ile ([Ile]) tended to decrease ( $p = 0.051$ ) in female patients on day 0; however, there was no significant difference in the groups of male patients. However, on day 7, plasma concentrations increased significantly compared to those on day 0 and tended to be higher than the values in the C group for both males and females. There was no significant difference in the plasma concentration of Leu ([Leu]) between the C and CAP groups on days 0 and 7 for either males or females. Although the plasma concentration of Val ([Val]) did not differ among the three groups in males, it decreased on day 0 in female patients. Figure 2 shows the plasma concentration ratios of the following BCAA combina-



**Fig. 1.** Plasma concentrations of branched-chain amino acids for control, day 0, and day 7. The horizontal solid lines indicate the mean values for each group. BCAA, branched-chain amino acid; C, control group; day 0, sample from bacterial pneumonia patients within 24 h after hospitalization; day 7, sample from bacterial pneumonia patients 6–8 days after hospitalization.

tions: [Ile] vs [Leu], [Ile] vs [Val], and [Leu] vs [Val], which were evaluated by the “b” value. The regression lines (the dashed lines in the figure) represent the regression lines of the C group, and a strong positive correlation ( $r>0.7$ ) was maintained for all combinations. Each [BCAA] of the C group and the “b” value of the regression lines ( $Y = aX + b$ ) are shown in Table 3. Each [BCAA] was significantly higher in males than in females; however, there was no difference in the “b” value between the male and female participants. Additionally, the distribution of each [BCAA] for both male and female patients was observed around the regression lines (dashed lines in the figure) of the C group (Fig. 2, day 0). On day 7, the [Ile] vs [Leu] and [Leu] vs [Val] ratios were largely distributed below the regression line of the C group. Figure 3 shows the comparison of the “b” values for the C and CAP groups on days 0 and 7 in male and female participants. In the [Ile] vs [Leu] ratio, there was no difference in the “b” values of the C group on day 0 compared to the CAP group for either male or female participants, although the value decreased on day 7. For the [Ile] vs [Val] ratio, the “b” values were lower on days 0 and 7 in the CAP group than in the C Group; however, this was only observed in the group of female participants. Among males, there was a significant difference only between days 0 and 7 in the CAP group. In the ratio of [Leu] and [Val], the change in the “b” value differed between the male and female participants. In the group of male participants, there was no difference between the C and CAP groups on day 0, although there was an increase in the “b” value on day 7. The “b” value decreased on day 0 in the female CAP group; however, this difference disappeared on day 7.

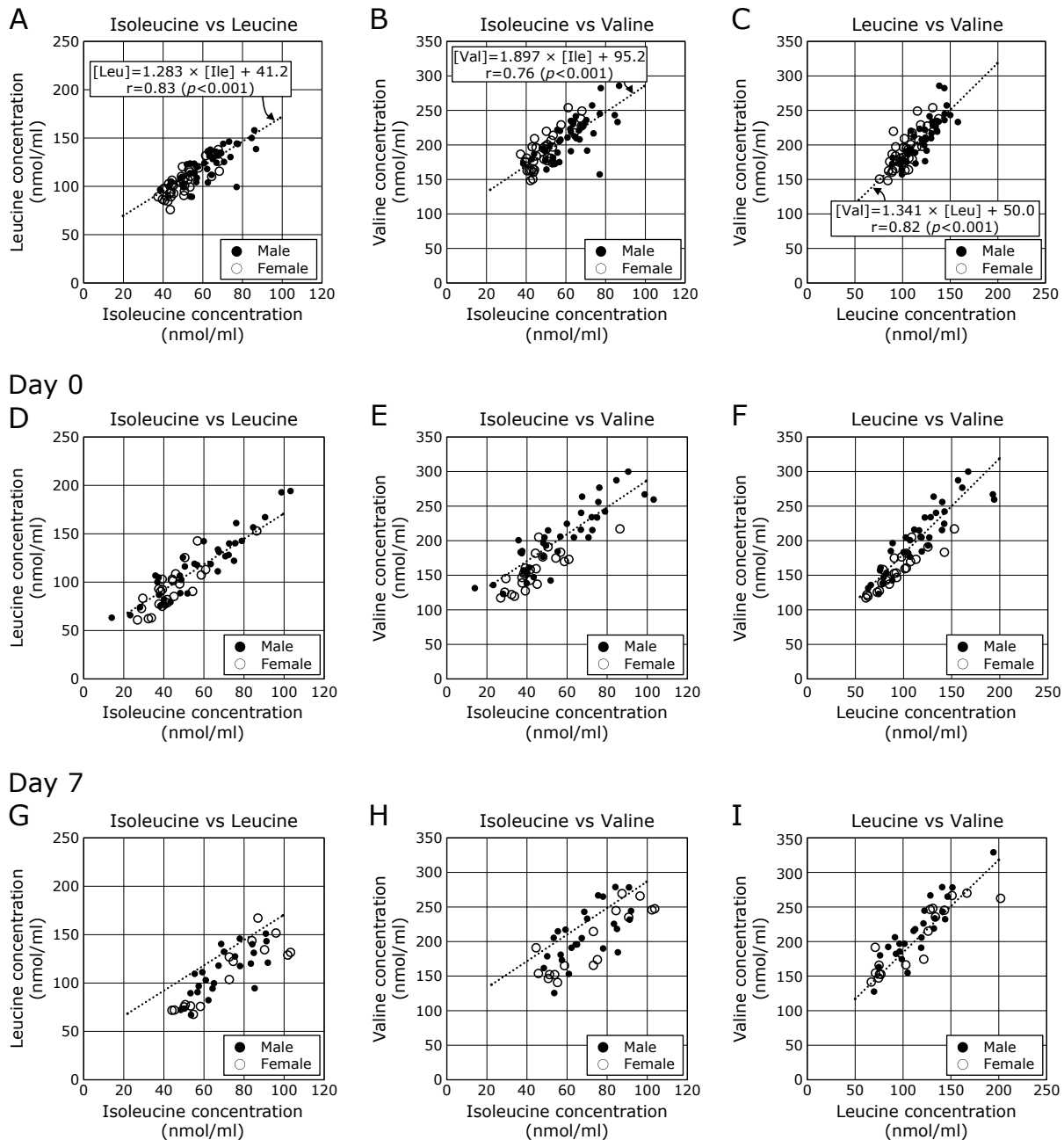
## Discussion

In this study, a proposed evaluation method was used to analyze novel changes in [BCAA] in bacterial pneumonia. The study results indicate a metabolic function maintaining the plasma concentration of leucine, starting from the acute phase and persisting through the convalescent phase of bacterial pneumonia.

The plasma concentration of each BCAA is strongly correlated with the concentrations of the other BCAAs, and this correlation is maintained even in diseased states.<sup>(19)</sup> Moreover, as the regression lines do not pass through the origin, when comparing the concentration of each group of BCAAs, the concentration ratios change depending on the specific BCAA concentrations. The [Ile] of healthy Japanese individuals is between 36.4 and 85.0  $\mu\text{M}$ .<sup>(20)</sup> Therefore, examining the regression line (Fig. 3) for a healthy person, assuming that [Ile] is 40  $\mu\text{M}$ , then [Leu] and [Val] would be 94 and 167  $\mu\text{M}$ , respectively, which indicates a ratio of 1:2.4:4.2. If [Ile] is 80  $\mu\text{M}$ , then [Leu] and [Val] would be 144 and 247  $\mu\text{M}$ , respectively, which implies a ratio of 1:1.8:3.1. As described above, even in healthy individuals, the relative ratios of each group of BCAAs vary depending on the concentrations being compared. Therefore, in this study, a new evaluation index was created by fixing the coefficient “a” of the slope of the regression line ( $Y = aX + b$ ) and subsequently calculating the “b” value from the [BCAA] values of each participant.<sup>(19)</sup>

Fever and IL-6 induction, which occur via prostaglandin E2 (PGE2), are part of the immune response during bacterial infections, such as pneumonia.<sup>(24,25)</sup> In primates, IL-6 is the major CRP inducer,<sup>(26)</sup> and IL-6 produces CRP from hepatocytes.<sup>(27)</sup>

## Control



**Fig. 2.** Correlation between plasma concentrations of each branched-chain amino acid (BCAA) in the control, day 0, and day 7 groups. Each correlation was calculated using the least-squares method; control group, A, B, and C; day 0, D, E, and F; and day 7, G, H, and I. Day 0, sample from bacterial pneumonia patients within 24 h after hospitalization; day 7, sample from bacterial pneumonia patients 6–8 days after hospitalization.

Although blood PGE<sub>2</sub> and IL-6 levels were not measured in this study, the measurement of changes in CRP levels may be a substitute for those levels. Therefore, as the fever had subsided and CRP values were low on day 7 (Table 2), PGE<sub>2</sub> production and IL-6 induction may have decreased. However, during the convalescent phase (day 7), some plasma amino acid concentrations did not return to the values observed in the healthy group, although day 7 was definitely in the convalescent phase.<sup>(20)</sup> The changes in the total [BCAAs] and each [BCAA] did not differ between the healthy group and patients in the convalescent phase, except for [Ile] in female participants (Fig. 1). However, comparisons of each amino acid concentration in terms of the

calculated “b” value showed differences between the CAP and C groups, except for the ratio of [Ile] and [Val] for males and [Leu] and [Val] for females (Fig. 3). Therefore, the changes in BCAA metabolism, which are often ambiguous when observing changes in each [BCAA], were ascertained by evaluating “b” values in this study.

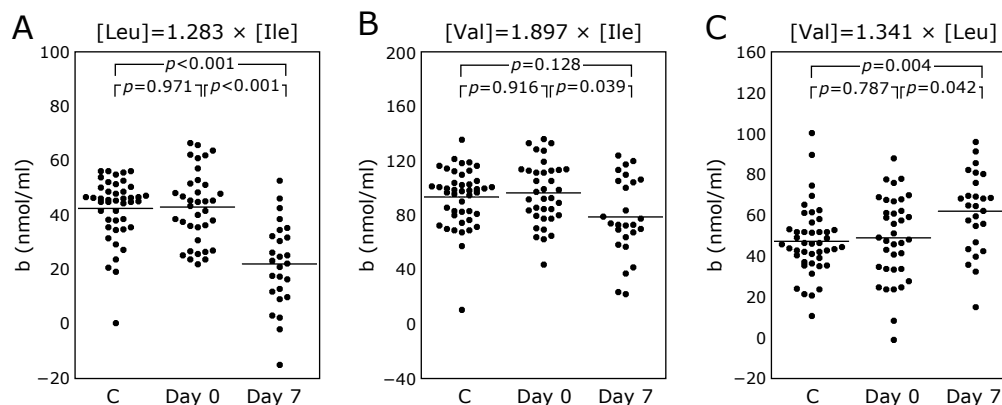
Skeletal muscle catabolism is enhanced at the onset of infectious diseases, and amino acids released from the muscles are used for gluconeogenesis.<sup>(28,29)</sup> In addition, muscles supply BCAAs, in the form of branched-chain  $\alpha$ -keto acids (BCKAs), to organs, such as the liver and kidneys, and these BCKAs are temporary metabolites that act as reservoirs for BCAAs.<sup>(30)</sup> The

**Table 3.** Plasma concentrations of branched-chain amino acids and the calculated “b” values

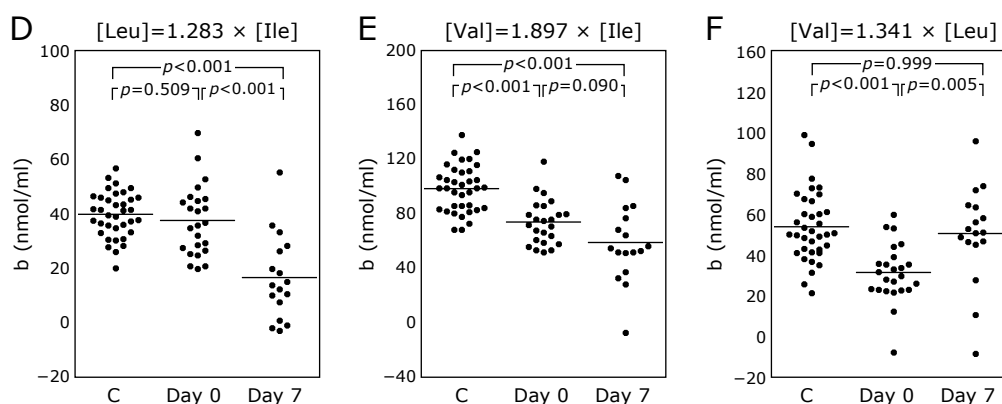
	Male (n = 44)	Female (n = 36)	p value
	Mean ± SD	Mean ± SD	
Isoleucine (nmol/ml)	62.32 ± 11.24	49.16 ± 8.35	<0.001
Leucine (nmol/ml)	122.29 ± 33.44	102.85 ± 13.90	<0.001
Valine (nmol/ml)	211.17 ± 29.83	191.31 ± 26.74	0.004
b = [Leu] – 1.283 × [Ile]	42.34 ± 11.38	39.79 ± 8.32	0.071
b = [Val] – 1.897 × [Ile]	92.93 ± 21.40	98.05 ± 17.05	0.368
b = [Val] – 1.341 × [Leu]	47.17 ± 16.78	53.38 ± 17.01	0.104

[Ile], plasma concentration of isoleucine; [Leu], plasma concentration of leucine; [Val], plasma concentration of valine.

### Male



### Female



**Fig. 3.** Distribution of “b” calculated from  $Y = aX + b$ . The regression line of  $Y = aX + b$  enabled the determination of “b” for each individual by fixing the value of “a” to that of the regression line of healthy participants and the replacement of X and Y with the BCAA plasma concentration of each individual. The values of “a” for Leu/Ile, Val/Ile, and Val/Leu were 1.283, 1.897, and 1.341, respectively (Table 3). The values of “b” for Leu/Ile, A and D; the values of “b” for Val/Ile, B and E; the values of “b” for Val/Leu, C and F. The dots represent the calculated “b” of each participant. C, control group; day 0, sample from bacterial pneumonia patients within 24 h after hospitalization; day 7, sample from bacterial pneumonia patients 6–8 days after hospitalization.

study results showed that the increase in phenylalanine and the hyper-catabolism of muscles may persist even during the convalescent phase (day 7).<sup>(20)</sup> The rate of BCAA metabolism is predominantly controlled by the muscular branched-chain amino acid aminotransferase (BCAAT) and hepatic and renal branched-chain  $\alpha$ -keto acid dehydrogenase (BCKAD).<sup>(5,30,31)</sup> In the postprandial state, BCAA metabolism is activated by BCAAT, and the release of glutamic acid, alanine, and BCKA from muscles is increased.<sup>(31)</sup> The plasma concentration of alanine is significantly reduced during the acute phase of

bacterial pneumonia, whereas the glutamic acid levels do not change.<sup>(20)</sup> In this study, there was no significant difference in [Leu] between the healthy group and the patient group on days 0 and 7 for either male or female participants (Fig. 1). This indicates the existence of a control mechanism to keep the [Leu] constant, regardless of changes in glycolysis and gluconeogenesis from amino acids during bacterial pneumonia. The adipose tissue is responsible for releasing 5% to 10% of all leucine that is released from body tissues,<sup>(32)</sup> and both renal<sup>(33)</sup> and muscular tissues may contribute to [Leu] homeostasis along

**Table 4.** The calculated “b” values from patients with fulminant hepatic failure in previous study<sup>(42)</sup>

	Control	Survivors	Non survivors
$b = [\text{Leu}] - 1.283 \times [\text{Ile}]$	62.9	62.9	128.7
$b = [\text{Val}] - 1.897 \times [\text{Ile}]$	186.8	120.3	121.3
$b = [\text{Val}] - 1.341 \times [\text{Leu}]$	117.3	48.5	76.3

[Ile], plasma concentration of isoleucine; [Leu], plasma concentration of leucine; [Val], plasma concentration of valine.

with the adipose tissue. Alternatively, Leu, unlike Ile and Val, is not used for gluconeogenesis,<sup>(34)</sup> which may explain the lack of change in [Leu] during bacterial pneumonia. Regarding the concentration ratios of each BCAA, [Leu] remains fixed, whereas the changes in [Ile] and [Val] are relative to [Leu]. The comparison of [Ile] and [Leu] showed similar changes for both males and females. Leu is the most frequently utilized BCAA in lymphocyte proliferation,<sup>(25)</sup> whereas Ile and Val suppress Leu metabolism.<sup>(14)</sup> Therefore, [Ile] could have relatively increased on day 7 compared to [Leu] and suppressed the metabolism of Leu in peripheral lymphocytes.<sup>(14)</sup> However, there was a sex-related difference in the individual changes in [Ile] and [Val], which indicated a sex-related difference in the metabolic response to infection. Ile enhances glucose uptake into muscles<sup>(35)</sup> and promotes glucose consumption.<sup>(36)</sup> Therefore, the increase in [Ile] on day 7 in female participants may have promoted glucose uptake into muscles. PGE2 induces BCAA-fueled heat production in the mitochondria of brown adipocytes, and Val oxidation, in particular, is enhanced.<sup>(10)</sup> Furthermore, BCAA metabolism differs according to the type of adipose tissue.<sup>(37)</sup> The decrease in [BCAA] and [Val] observed in female participants (which was not observed in males) of the CAP group on day 0 may be attributed to differences between males and females in the amount of skeletal muscle and adipose tissue, which are sources of BCAAs.<sup>(38,39)</sup> In addition, sex-related differences in these [BCAA] changes may be related to sex-related differences in the immune response during an infection.<sup>(40,41)</sup>

The present study has some limitations. The value of the intercept (b) with respect to the Y-axis of the regression line ( $Y = aX + b$ ) of each amino acid ratio easily changed in response to the slope (a). Therefore, to compare the intercept (b) for various diseases in future research, it would be necessary to analyze the BCAA obtained from a larger number of healthy individuals. In addition, there may be racial differences in slope (a), which requires further research. Further study is required to investigate cytokines controlling amino acid metabolism during bacterial pneumonia. Additionally, further research is required to determine the mechanism underlying the maintenance of the cross-correlation of each [BCAA], even in disease states, and the mechanism of regulation of intercept (b). All patients in this study appropriately recovered from bacterial pneumonia; therefore, the contribution of “b” value to the outcome of pneumonia could not be examined. The “b” values calculated from a previous study<sup>(42)</sup> showed the possibility of predicting the prognosis of severe acute hepatitis (Table 4). However, a further study examining the clinical significance of the measurement of plasma amino acid concentration of BCAAs is required.

In conclusion, the study results indicate a metabolic function that maintains the plasma concentration of leucine, starting from the acute phase and persisting through the convalescent phase of bacterial pneumonia. Furthermore, there was a sex-related difference in the [BCAA] ratios containing valine, which indicates a sex-related difference in the metabolic response to bacterial infection. The evaluation of [BCAA] by intercept (b) of the

regression line in this study possibly reveals the changes in BCAA metabolism, which are ambiguous when observing changes in each [BCAA].

## Author Contributions

HI had full access to the study data, takes full responsibility for the integrity of the data and the accuracy of the analysis, contributed to the study design, wrote the manuscript, conducted data analysis, drafted and revised the manuscript, and agrees to be accountable for all aspects of the work.

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## Abbreviations

BCAA	branched-chain amino acid
[BCAA]	plasma concentration of branched-chain amino acid
BCKA	branched-chain $\alpha$ -keto acid
[BCKA]	plasma concentration of branched-chain $\alpha$ -keto acid
BUN	blood urea nitrogen
C	control group
CAP	community-acquired pneumonia
CRP	C-reactive protein
day 0	bacterial pneumonia patients within 24 h after hospitalization
day 7	bacterial pneumonia patients 6–8 days after hospitalization
eGFR	estimated glomerular filtration rate
HbA1c	glycated hemoglobin
IL-6	interleukin 6
Ile	isoleucine
[Ile]	plasma concentration of isoleucine
Leu	leucine
[Leu]	plasma concentration of leucine
LPS	lipopolysaccharides e
PGE2	prostaglandin E2
Val	valine
[Val]	plasma concentration of valine
WBC	white blood cell count

## Conflict of Interest

No potential conflicts of interest were disclosed.

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