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Sodium Phenylbutyrate Decreases Plasma Branched-Chain Amino Acids in Patients with Urea Cycle Disorders

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Abstract

Sodium phenylbutyrate (NaPBA) is a commonly used medication for the treatment of patients with urea cycle disorders (UCDs). Previous reports involving small numbers of patients with UCDs have shown that NaPBA treatment can result in lower plasma levels of the branched-chain amino acids (BCAA) but this has not been studied systematically. From a large cohort of patients (n=553) with UCDs enrolled in Longitudinal Study of Urea Cycle Disorders, a collaborative multicenter study of the Urea Cycle Disorders Consortium, we evaluated whether treatment with NaPBA leads to a decrease in plasma BCAA levels. Our analysis shows that NaPBA use independently affects the plasma BCAA levels even after accounting for multiple confounding covariates. Moreover, NaPBA use increases the risk for BCAA deficiency. This effect of NaPBA seems specific to plasma BCAA levels, as levels of other essential amino acids are not altered by its use. Our study, in an unselected population of UCD subjects, is the largest to analyze the effects of NaPBA on BCAA metabolism and potentially has significant clinical implications. Our results indicate that plasma BCAA levels should to be monitored in patients treated with NaPBA since patients taking the medication are at increased risk for BCAA deficiency. On a broader scale, they could open avenues to explore NaPBA as a therapy in maple syrup urine disease and other common complex disorders with dysregulation of BCAA metabolism.

Keywords

urea cycle disorder; sodium phenylbutyrate; branched-chain amino acids

1. INTRODUCTION

Sodium phenylbutyrate (NaPBA)[1] is a nitrogen-scavenging agent that is used in the routine management of patients with urea cycle disorders (UCDs) [1–4]. Phenylacetate, the active metabolite generated from NaPBA, conjugates glutamine to form phenylacetylglutamine

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which is excreted in the urine. Thus, NaPBA provides an alternative route for the disposal of waste nitrogen by diverting waste nitrogen from entering the urea cycle and is used to prevent hyperammonemia. However, NaPBA has diverse biological effects including histone deacetylase inhibition, reduction of endoplasmic reticular stress, modulation of protein phosphorylation, inhibition of adipogenesis, and improvement of glucose homeostasis [5–7]. NaPBA also has been noted to affect the metabolism of the branched-chain amino acids (BCAAs) [8].

Low levels of plasma BCAA in patients with UCDs were first noted in a long-term follow-up of 24 patients with citrullinemia who participated in the early studies of the nitrogen-scavenging agents (NaPBA, sodium phenylacetate, and sodium benzoate) [9]. Small studies in control subjects and patients with UCDs have since demonstrated that low plasma BCAA levels are associated with use of NaPBA [8, 10, 11]. In contrast, decreased BCAA levels have not been observed with sodium benzoate [11]. An initial survey of plasma BCAA levels in patients with UCDs (n=183) enrolled in the Longitudinal Study of Urea Cycle Disorders, a natural history study conducted by the Urea Cycle Disorders Consortium, demonstrated that patients administered NaPBA had lower BCAA levels relative to those who were not on the medication [12, 13]. However, patients with disorders that result in a proximal blockade in ureagenesis, those with frequent metabolic decompensations, and those with more severe protein restriction, are more likely to be prescribed NaPBA. Detailed analyses accounting for covariates that could confound these observations have not been conducted. In the present study, we evaluated a large population of patients with a variety of UCDs to test whether NaPBA use was independently associated with low BCAA levels even when accounting for potential confounders. In addition, we assessed whether the dose of NaPBA correlates with BCAA levels and whether low BCAA levels increase the risk of hyperammonemia as correlations between these factors, if they exist, could have implications on the management of patients with UCDs.

2. MATERIALS AND METHODS

2.1 Patient population and data collection

Data were collected as part of the Longitudinal Study of Urea Cycle Disorders (ClinicalTrials.gov NCT00237315) [12]. This is a natural history study conducted by the Rare Diseases Clinical Research Network's (RDCRN) Urea Cycle Disorders Consortium (UCDC) that includes 14 academic center sites in the United States, Canada, and Europe [12]. Data from all centers were collected in a standardized format as detailed in the manual of operations and were entered into the electronic database maintained by the Data Management and Coordinating Center of the RDCRN. The data obtained from the enrollment visit were used in the analyses.

We collected data from 611 subjects. Fifty-eight subjects were excluded from the analysis because BCAA levels were not available at the enrollment visit or because of data entry

[1]**Abbreviations:** NaPBA - sodium phenylbutyrate; UCD - urea cycle disorder; BCAA - branched-chain amino acids; RDCRN - Rare Diseases Clinical Research Network; GLM - generalized linear model; OTCD - ornithine transcarbamylase deficiency; BCKDK - branched-chain ketoacid dehydrogenase kinase; BCKDC - branched-chain ketoacid dehydrogenase complex; MSUD - maple syrup urine disease

errors. Fifteen subjects who were taking BCAA supplements at enrollment were also included in the primary analysis. For each of the remaining 553 subjects, the following data were collected from the initial enrollment visit: UCD diagnosis, onset of disease (neonatal *vs* later onset), age at enrollment, gender, daily reported protein intake (g/kg/day), NaPBA use, and plasma levels of BCAA, other essential amino acids, albumin, and prealbumin. Plasma samples for amino acid analyses were collected after a 3-hour fast and before administration of the nitrogen-scavenging medication. Dietary data were collected and analyzed based on a 3-day diet; when such data were not available, a 24-hour recall was utilized. The laboratory assessments were performed at the local CLIA-certified laboratories. For the analysis of BCAA deficiency, the following thresholds for normal range were used: <30 μMol/L for leucine, <10 μMol/L for isoleucine, and <70 μMol/L for valine.

2.2 Hyperammonemia analysis

Symptomatic hyperammonemia episodes were defined in the manual of operations and recorded when plasma ammonia was greater than 100μM and required an ER visit, hospitalization, or an unscheduled clinic visit. Ammonia levels were measured at the local facilities where patients presented for evaluation of hyperammonemia. The number of hyperammonemic episodes was recorded based on patient report which was confirmed by review of medical records whenever possible. To analyze whether low plasma BCAA levels conferred a higher risk for hyperammonemia, we calculated the odds ratio of a hyperammonemic event occurring within 12 months of enrollment in subjects with plasma levels of at least two of the three BCAAs in the lowest quartile vs. those with at least two of the three BCAAs in the highest quartile.

2.3 Statistical analysis

Two-sample comparisons were performed using Mann-Whitney U test. Chi-square analysis was used for comparison of proportions. These statistical analyses and calculations of odds ratios with confidence intervals were performed using GraphPad Prism v 6.03.

To account for the covariates that influence BCAA and other essential amino acid levels, we performed generalized linear model (GLM) analysis. For each amino acid, data was available for all other covariates in 333 study subjects and these subjects were included in subsequent GLM analysis. The plasma levels of each BCAA was the dependent variable while the continuous variables – age and daily protein intake, and categorical variables - gender, type of UCD, onset of presentation, NaPBA use, plasma albumin and prealbumin levels were independent variables. The laboratory tests for patients enrolled in the Longitudinal Study of Urea Cycle Disorders are analyzed at different laboratories. Thus, we used standard estimates of normal ranges for the GLM analysis. Albumin was converted into a categorical variable (normal versus abnormal) based on the following normal values for age (0 – 30 days, 2.9–5.5 (g/dL); 1 – 3 months, 2.8–5.0; 4 – 11 months, 3.9–5.1 and > 1 year, 3.7–5.5 g/dL). Prealbumin was converted to a categorical variable (normal vs abnormal) based on the following: (0–6 days, 4–20 mg/dL; 7–41 days, 8–25; > 42 days, 18–44 mg/dL). The analysis was completed using the GLM function in R project for Statistical computing (http://www.R-project.org/) [14]. Leucine, isoleucine, valine and other essential amino acids did not fit a normal distribution, thus these variables were fit to a gamma

distribution. In GLM, the analysis was performed with the gamma family and inverse link function. The complete model was compared to the null model. The models are listed as follows:

Null model:

Plasma leucine= μ+ A1 (age) + A2 (gender) + A3 (onset) + A4 (UCD diagnosis) + A5 (protein intake) + A6 (plasma prealbumin) + A7 (plasma albumin)

Full model:

Plasma leucine= μ+ A1 (age) + A2 (gender) + A3 (onset) + A4 (UCD diagnosis) + A5 (protein intake) + A6 (plasma prealbumin) + A7 (plasma albumin) + A8 (NaPBA use)

This analysis was repeated for isoleucine, valine, and other essential amino acids. Significance of the coefficients was calculated in R. Significance of the model comparison was calculated using the difference of the −2(ln_likelihood), and this was compared to a chi-square distribution with one degree of freedom (given 9 degrees of freedom in the full model versus 8 degrees of freedom in the null model). Interaction between significant independent variables (coefficient $p < 0.05$) was examined by comparing the full model to the full model with the addition of an interaction term. GLM was also used to examine the effect of NaPBA dose on BCAA levels. For subjects weighing less than 20 kg (n=83), NaPBA dose was expressed in mg/kg. The participants were further subdivided by dose into three groups [<250 mg/kg (n=30), 250–500 mg/kg (n=39), and >500 mg/kg (n=14)]. For subjects weighing more than 20 kg (n=90), NaPBA dose was expressed in g/m^2 body surface area. Analogous to the participants weighing less than 20kg, we subdivided participants into three groups [NaPBA dose <5 g/m^2 (n=16), 5–10 g/m^2 (n=44), and >10 g/m^2 (n=30)]. Separately, for subjects weighing less than 20 kg or more than 20 kg, the null model (as above) was compared to a model that included the independent variables in the null model and an additional independent variable for dosage group. In this study, 39 statistical tests were performed, and we corrected for multiple comparisons in order to reduce type 1 error. We used the conservative Bonferroni correction and at an α of 0.05 an uncorrected p-value of 0.00128 was treated as significant.

3. RESULTS

3.1 Study Population

The study population is summarized in Table 1. Of the 553 subjects, 212 (38%) were on NaPBA and 341 (62%) were not taking the medication at the time of enrollment. There were differences in the baseline characteristics between the two groups. As expected, the patients taking NaPBA were younger ($p_{corrected}<0.005$) and more likely to have neonatal-onset disease ($p_{corrected}<0.005$). The distribution of diagnoses also differed between the two groups. For instance, a higher percentage of female patients with ornithine transcarbamylase deficiency (OTCD) was observed in the group not taking NaPBA ($p_{corrected}=0.039$) in contrast to a trend for a higher percentage of male patients with OTCD in the group taking NaPBA ($p_{uncorrected}=0.01$).

3.2 NaPBA use is associated with lower plasma BCAA levels

Our initial analysis revealed that the plasma leucine, valine, and isoleucine levels were significantly lower in the subjects taking NaPBA (Mann-Whitney Test, $p_{corrected}<0.005$ for each amino acid) (Table 2). Because a variety of covariates could explain the differences observed, we reanalyzed the data using a GLM comparison to evaluate covariates. Of the 7 variables, age, protein intake, and NaPBA use significantly contributed to the level of leucine, isoleucine and valine. Age and protein intake positively correlated with levels of each of the three BCAAs whereas NaPBA use was negatively correlated with BCAA levels (Supplementary Table 1). A comparison of the null vs. full model revealed significant effects of NaPBA on plasma levels of leucine ($p_{corrected}$= 2.41e–07), isoleucine ($p_{corrected}$= 2.52e–05) and valine ($p_{corrected}$= 2.25e–04) which demonstrates that when the other variables are accounted for, there is a significant independent correlation between the levels of BCAAs and the use of NaPBA. Evaluation of interaction terms indicated that they did not improve the model.

3.3 NaPBA does not affect plasma levels of other essential amino acids

If the decreased BCAA levels in subjects taking NaPBA were a result of protein restriction, the levels of other essential amino acids would also be lower. To provide further evidence that NaPBA specifically correlated with BCAA levels, we compared the effects of NaPBA on the plasma levels of other essential amino acids. Using the same GLM, we found no significant effects of NaPBA on the levels of threonine ($p_{uncorrected}$=0.46), phenylalanine ($p_{uncorrected}$=0.06), methionine ($p_{uncorrected}$=0.12), histidine ($p_{uncorrected}$=0.08), and lysine ($p_{uncorrected}$=0.2) (Figure 1). Thus NaPBA use specifically correlated with decrease in plasma BCAA, without similar correlations in other essential amino acids.

3.4 NaPBA increases the risk for BCAA deficiency

BCAA levels at the lower end of the normal range may not have many clinical implications. However, BCAA deficiency may be clinically significant and could potentially impact metabolic status in patients with UCDs. Thus, we evaluated whether subjects taking NaPBA were at higher risk for deficiency of any of the three BCAAs. Because the samples were processed at different laboratories with varying sensitivities and normal ranges, we used a conservative estimate for defining deficiency of individual BCAA (leucine: < 30 μMol/L, isoleucine < 10 μMol/L, and valine < 70 μMol/L). In subjects taking NaPBA the odds ratio for deficiencies of leucine, isoleucine, and valine were 8.1 (95% confidence interval (CI) of 3.0 – 21.7, $p_{corrected}$ <0.005), 3.3 (CI of 0.8 to 13.2, $p_{uncorrected}$ = 0.0943), and 7.7 (CI 2.6 – 23.2, $p_{corrected}$ <0.005), respectively. These results indicate that NaPBA use is associated with an increased risk for BCAA deficiency.

3.5 Dosage of NaPBA does not determine BCAA levels

To evaluate whether the decreased BCAA levels correlated with the dose of NaPBA, we conducted further analysis using GLM. For this analysis, subjects being supplemented with BCAA (n=15) and patients for whom dosing was not available (n=40) were excluded and the remaining 173 subjects were categorized based on body weight. Our analyses did not

show an independent effect of the dose of NaPBA on the levels of BCAA. Thus, we did not find evidence that higher doses of NaPBA correlated with lower levels of BCAA.

3.6 Low BCAA and hyperammonemic episodes

One concern with low plasma BCAA levels is that if limiting, they could lead to catabolism, especially in the setting of stress and thus increase risk for metabolic decompensation. To test whether a higher percentage of patients with the lowest BCAA levels had episodes of hyperammonemia in the one year following the enrollment visit as compared to patients with higher BCAA levels, we divided the patients taking NaPBA into two groups: one with at least two of three plasma BCAAs in the lowest quartile (n=52) and the other with at least two of three BCAA levels in the highest quartile (n=51) (Supplementary Table 2). The odds ratio for having at least one hyperammonemia episode in the year following enrollment was 1.63 (CI 0.72–3.69, $p_{uncorrected}$=0.30) for patients with low BCAA vs. high BCAA. If the groups were to be subdivided to only include patients with proximal UCDs (i.e. OTCD, CPS 1 deficiency, and NAGS deficiency), the odds ratio was 2.16 (CI is 0.72-6.47, $p_{uncorrected}$ = 0.18). These results have to be interpreted with caution as multiple factors including many environmental factors, contribute to hyperammonemia episodes. A longitudinal analysis in individual patients could help provide a more definitive answer as to whether low BCAA increases risk for hyperammonemia.

4. DISCUSSION

Using data from a large cohort of subjects with UCDs who are managed at metabolic centers across the United States and Europe, we have demonstrated lower plasma BCAA levels in subjects taking NaPBA as a nitrogen-scavenging agent. Our large sample size allowed for a comprehensive analysis of multiple covariates and demonstrated that the association between NaPBA use and lower plasma BCAA levels is independent of covariates such as age, gender, age of onset, UCD diagnosis, and nutritional measures, such as prealbumin and protein intake. Furthermore, when these covariates are taken into account, the finding that there is no significant association between NaPBA use and the plasma levels of other essential amino acids provides further evidence that the lower levels of BCAA are not the result of generalized protein deficiency. We did not find evidence of a relationship between dosage of NaPBA and BCAA levels, however these analyses were completed on a smaller subset of subjects, which decreases the power of this analysis. These results raise two pertinent questions: 1) what are metabolic consequences of the lowering of BCAA in patients with UCDs? 2) can NaPBA be used to modulate BCAA metabolism in other conditions?

Although the consequences of decreased levels of BCAA in patients with UCDs are unclear, it has been hypothesized that decreased BCAA levels may be associated with increased risk for metabolic decompensation as low levels of BCAA have been observed in patients presenting with hyperammonemia [15]. Low BCAA levels could potentially promote catabolism in the setting of environmental stress, such as intercurrent illness. To date, no longterm studies of BCAA levels and risk for metabolic decompensation in UCD patients taking NaPBA have been performed. Our study did not find evidence of an association

between plasma BCAA and hyperammonemia episodes in the following one year; however, the analysis did not account for potential confounders including environmental stressors. In addition only a small subset of the participants met criteria to be included in this analysis, thus reducing power to detect a significant correlation. Furthermore, we could not assess whether patients with low BCAAs are at higher risk in the days to weeks after the measurement, the time period that may be most relevant. Interestingly, a recent study revealed that plasma BCAA levels tended to be low in patients with UCDs at the time of admission for a hyperammonemic episode [15]. However, in these patients, other essential amino acids were also low, and none of the patients were taking NaPBA [15]. Longitudinal analyses involving BCAA levels and hyperammonemia episodes are necessary to test whether the decrease in BCAA, especially in those with BCAA deficiency, increases risk for hyperammonemia. Another concern of long-term BCAA deficiency is that it could lead to detrimental neurologic outcomes even in the absence of metabolic decompensation [16, 17]. Patients with biallelic mutations in branched-chain ketoacid dehydrogenase kinase (BCKDK) and low plasma BCAA have neurocognitive phenotypes [16, 17]. Corroborating these human data, a mouse model of BCKDK deficiency exhibits seizures, tremors, lower plasma and brain levels of BCAAs and elevations in other large neutral amino acids [17].

BCAAs are catabolized via the branched-chain ketoacid dehydrogenase complex (BCKDC). The BCKDC consists of an E1 decarboxylase component (which contains E1α and E1β subunits), an E2 transacylase component (which has 24 identical subunits), and an E3 component (homodimeric structure). *In vitro* and animal studies have demonstrated that NaPBA prevents phosphorylation of the E1α subunit of the BCKDC and thus increases the activity of the complex [8, 18]. This effect of NaPBA could be potentially utilized to modulate dysregulated BCAA metabolism in disease states, such as maple syrup urine disease (MSUD). In fact, pilot studies have shown that NaPBA lowers BCAA levels in control subjects and a subset of patients with MSUD [8]. A randomized double blind placebo controlled study is underway to investigate whether NaPBA may be an effective therapy for patients with MSUD (ClinicalTrials.gov Identifier: NCT01529060). Abnormal BCAA metabolism has been implicated in pathogenesis of more common disorders including insulin resistance, hepatic disease, and cancer. Thus, insights into effects of NaPBA on BCAA metabolism obtained from the study of rare Mendelian diseases could potentially have an impact on the treatment of common complex diseases.

5. CONCLUSIONS

Overall, our results demonstrate that NaPBA decreases plasma BCAA in patients with UCDs. Our data, together with published data showing low BCAA levels at the time of admission for metabolic decompensation [15], suggests that BCAA levels should be closely monitored especially since patients taking NaPBA may be at increased risk for BCAA deficiency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We assessed the association between sodium phenylbutyrate (NaPBA) use and plasma branched-chain amino acids (BCAA) in patients with Urea cycle disorders (UCDs)
- Even when accounting for multiple covariates, use of NaPBA is independently associated with lowering of branched-chain amino acids
- NaPBA use increases risk for branched-chain amino acid deficiency
- Our results suggest that BCAA levels need to be monitored in patients with UCDs who are treated with NaPBA

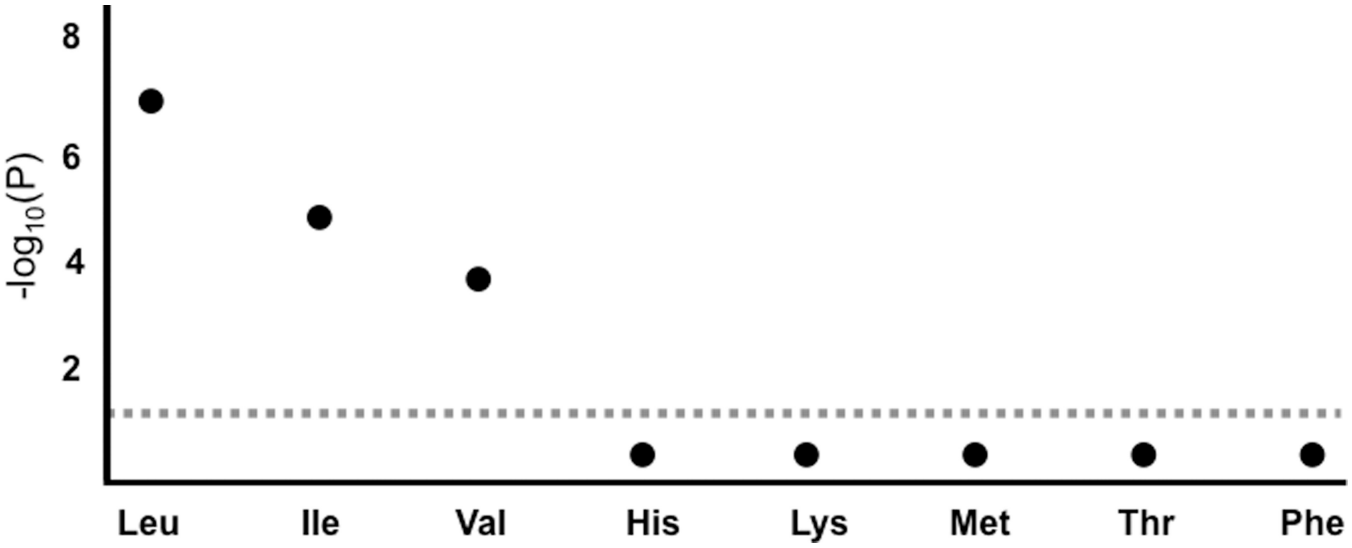

Figure 1. NaPBA specifically decreases plasma BCAA but not other essential amino acids

The figure depicts the logarithmic p-values from the GLM analysis for association between NaPBA use and plasma amino acid levels. The corrected p-value is plotted for each essential amino acid. The dashed line represents the level at which P would correspond to a value of 0.05 when corrected for multiple comparisons. Whereas the p-values for BCAA levels are very significant, those of other essential amino acids do not reach statistical significance.

Table 1

Baseline Characteristics of Subjects

Baseline characteristics are provided for the study population. For protein intake and laboratory values, the mean with standard deviation is listed. For age, the median with range is listed. P-values have been corrected for multiple testing using the Bonferroni correction.

	Patients Taking NaPBA	Patients Not Taking NaPBA	$p_{corrected}$ value
Total number	212	341	
Gender			NS
Male	41 % (86)	30 % (102)	
Female	59 % (126)	70 % (239)	
Age	8.3 years (0 – 68.2 years)	14.4 years (0 – 71.5 years)	< 0.005
Onset			<0.0 05
Neonatal	36 % (77)	19 % (66)	
Non-Neonatal	64 % (135)	81 % (275)	
Diagnosis			
CPS	3 % (7)	2 % (8)	N S
NAGS	0.1 % (2)	0.3 % (1)	NS
OTC (Female)	35 % (75)	47 % (169)	0.039
OTC (Male)	23 % (48)	13 % (44)	NS
ASS	20 % (42)	11 % (37)	NS
ASL	11 % (23)	18 % (63)	NS
ARG	5 % (11)	2 % (8)	NS
HHH	2 % (4)	0.9 % (3)	NS
Citrin	0 % (0)	0.6 % (2)	NS
Unspecified	0 % (0)	2 % (6)	NS
Protein Intake (g/kg) Labs	1.03 +/− 0.5	1.20 +/− 0.8	NS
Prealbumin (mg/dL)	23.2 +/− 6.6	22.2 +/− 6.3	NS
Albumin (g/dL)	4.0 +/− 0.6	4.1 +/− 0.5	NS
Receiving BCAA Supplement	7 % (14)	0.3 % (1)	<0.005

Table 2

Branched chain amino acid levels in patients taking NaPBA (n=212) vs. patients not taking NaPBA (n=341)

The medians and interquartile ranges are provided for each BCAA. The p-value has been corrected for multiple testing using the Bonferroni correction.

	NaPBA	No NaPBA	$P_{corrected}$ value
Leucine (μMol/L)	60 (40 – 85)	95 (72 – 121)	<0.005
Valine (μMol/L)	128 (92 – 169)	176 (142 – 217)	<0.005
Isoleucine (μMol/L)	31 (22 – 49)	49 (36 – 65)	<0.005