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Branched chain amino acids as a parameter for catabolism in treated phenylketonuria

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Summary. This study was performed to study an association between nutritional status on one hand and BCAA- and Phe-concentrations on the other hand in PKU patients free of infection. AA profiles from 70 PKU patients were measured. 9 patients (subgroup I) with elevated Phe- and BCAA-concentrations as well as 23 patients (subgroup II) with only elevated Phe-levels were included. Dietary records were obtained from both groups; low caloric intake in subgroup I was increased with Duocal® or *p-am* ANAMIX® without modifying total protein- and Phe-intake. AA profiles were controlled after 2 weeks.

Additionally, we investigated AA profiles from 26 liver transplanted patients with increased carbohydrate and caloric intake as an example for anabolism.

In subgroup I Phe- and Isoleu-concentrations decreased sign. after dietary intervention. Leu, Val and Tyr levels decreased not sign. Initial Phe-levels correlated negatively with protein and caloric intake.

BCAA concentrations of liver transplanted patients receiving high amounts of carbohydrates were in the lower range of normal.

Increased caloric intake lowered most of the elevated Phe- and BCAA-concentrations.

Keywords: PKU – BCAA – Catabolism – Diet – Compliance

Abbreviations: PKU, Phenylketonuria; BCAA, branched chain amino acids; AA, amino acids; wk, week; Phe, Phenylalanine; Isoleu, Isoleucine; Val, Valine; Leu, Leucine; Tyr, Tyrosine; sign., significantly

Introduction

Phenylketonuria (PKU; McKusick #261600) is an inherited disorder of Phe catabolism characterized by an inability to normally metabolize Phe to Tyr. Therapy consists of restricting dietary Phe to an amount that results in near normal plasma Phe-concentrations. Nutritional control can be accomplished through the use of Phe-free elemental medical food combined with natural protein to supply prescribed amounts of Phe.

Elevated BCAA have been reported to reflect catabolism (Arfvidsson et al., 1997; Biolo et al., 1995; Schauder et al., 1983). Without adequate energy intake amino acids are used for energy purposes. Branched chain amino acids are among the amino acids most sensitive to altered systemic insulin levels (De Barnola, 1965; Zieneman et al., 1966). Turnover of endogenous peptide-bound pools contributes to the plasma Phe pool. When nutrition is inadequate, protein catabolism occurs and BCAA's and free Phe are released.

As a consequence, plasma Phe values will initially rise in PKU patients without adequate energy intake in early negative nitrogen balance. Failure to recognize this phenomenon can confound treatment of PKU patients during episodes of inadequate nutrition.

A parameter is desirable to distinguish between dietary non-compliance and metabolic catabolism (defined as inadequate energy intake, based on the D.A.CH – *German, Austrian and Swiss working group for nutrition* – recommendations from 2000 associated with elevated plasma BCAA's) in treated PKU patients. Tyr is sometimes used as a parameter of catabolism in PKU. As Tyr is the product of the enzyme reaction deficient in PKU it does not a priori seem to be very sensitive in our opinion. Ketonuria can only be observed in *overt* catabolism and thus is a weak parameter to detect *latent* catabolism in treated PKU patients.

Methods like postabsorptive leucine metabolism as a marker of whole-body protein metabolism, measured by using a primed L-[1-^{13}C]leucine are described in children with chronic renal failure to measure protein metabolism (Boirie et al., 2000). Prealbumin has also been described

as an indicator of marginal malnutrition and starvation in treated PKU (Shenton et al., 1983). There are several studies describing that leptin – an adipocyte produced hormone- is strongly correlating to circulating levels of insulin (Chapman et al., 1997). Schulpis et al. could show the absence of a relation of leptin with the energy intake of PKU patients on "loose diet" (Schulpis et al., 2000). This is probably due to the dysregulation of their neurocrine system as shown by altered catecholamine levels. The same group described morning preprandial plasma ghrelin concentrations in patients with PKU (Schulpis et al., 2004). Ghrelin was recently described as a gastrointestinal hormone that is elevated in the fasting state. In poorly controlled PKU patients ghrelin levels did not correlate with energy intake.

Those methods and markers mentioned above do not seem to be practicable as routine parameters to control nutritional status in PKU patients.

This study was designed to detect a possible association between caloric intake and nutritional status on one hand and BCAA- and Phe-concentrations in plasma on the other hand in patients with classical PKU under Phe-restriction and free of infection.

Materials and methods

Patients' blood samples were drawn by venipuncture in the hospital's outpatient clinic or at the local pediatrician's office. AA profiles from 70 PKU patients were analyzed with an amino acid autoanalyzer (LC 3000, Biotronik).

The study design is summarized in Fig. 1. Patients with elevated age-adjusted Phe-levels as judged by the German recommendations for PKU (Burgard et al., 1998) were included in the study. They were divided into two groups. Those in subgroup II ($n = 23$) had normal BCAA-levels; low dietary compliance was suspected and Phe-intake was slightly reduced. And those in subgroup I ($n = 13$; 9 agreed to be studied – 6 female and 3 male age range 3–30 years) had elevated BCAA-concentrations; catabolism was assumed and energy intake was increased.

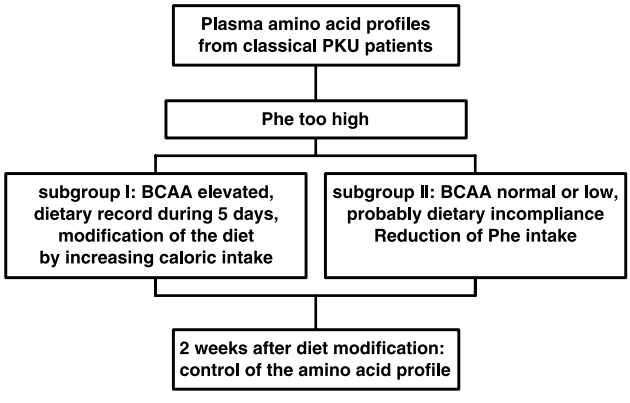

Fig. 1. Study design

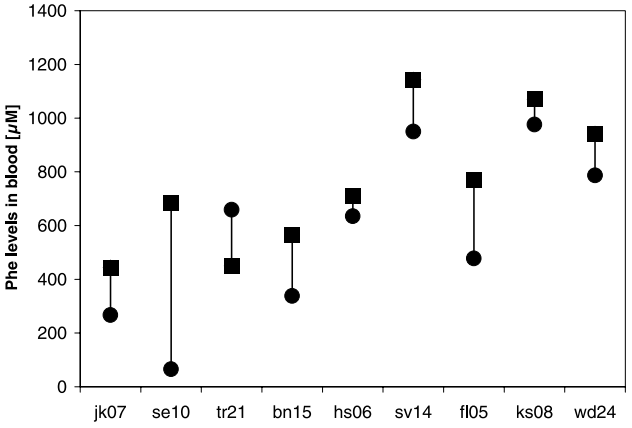

Fig. 2. Phe levels of subgroup I under dietary intervention: Phe concentrations ■ before and ● after caloric increment

Nutrient intake, based on 5 day-dietary records were calculated by the Prodi 4.5 LE 2001 software. Subgroup I patients with low caloric intake (based on the D.A.CH – *German, Austrian and Swiss working group for nutrition* – recommendations from 2000) were instructed to increase their caloric intake with *Duocal*® (mixture including per 100 g: 492 kcal, carbohydrates 72.2 g, fat 22.3 g) or *p-am ANAMIX*® (Phe-free amino acid mixture including per 100 g: 374 kcal, carbohydrates 34 g, protein: 29 g, fat 13.5 g). Total amounts of protein- and Phe-intake had not been modified. The effect of dietary intervention was determined after 2 weeks by reanalyzing plasma amino acid profiles (Fig. 2).

Because of special sample processing, initial insulin levels and those after dietary intervention had not been measured.

Additionally, we investigated plasma amino acid profiles from 26 liver transplanted patients with an increased carbohydrate and caloric intake ($\sim$16 g/kg per d, $\geq$120 kcal/kg per d) as an example for anabolism.

Data from amino acid levels were compared and Pearson-correlations of different parameters were calculated using Student's *t*-test for paired data. The level of significance was fixed for a p-value ≤ 0.05.

Results

9 PKU patients had Phe-concentrations above the age-adjusted therapeutic level as laid down in the German recommendations for PKU (Burgard et al., 1998) combined with elevated BCAA-levels as a sign for catabolism associated with normal Tyr-levels, no ketonuria (subgroup I). 23 PKU patients had elevated Phe-levels without BCAA-elevation (subgroup II).

The initial mean blood Phe-value for all patients included in subgroup I was 753 ± 254 μmol/l (Table 1). Subsequent means of Phe-concentrations 2 weeks after starting dietary intervention with additional caloric intake decreased to 573 ± 312 μmol/l ($p = 0.04$), (Figs. 2 and 3). A statistically not significant decrease of the mean Tyr-value from 92 ± 50 before to 75 ± 33 μmol/l after dietary intervention occurred. Mean blood Isoleu, Leu and Val levels revealed a different pattern of response, the strongest effect could be seen in the decrease of Isoleu

Table 1. Details of subgroup I patients with elevated Phe and BCAA-levels included in our study (*D.A.CH:* German, Austrian and Swiss working group for nutrition – recommendations from 2000)

Patient	Age [yr]	Sex	Phe intake [mg/kg/d]	Protein intake [g/kg/d]	D.A.CH recomm. protein [g/kg/d]	Energy intake [kcal/kg/d]	D.A.CH recomm. energy [kcal/kg/d]	Phe 0 wk [μM]	Phe 2 wks [μM]	Isoleu 0 wk [μM]	Isoleu 2 wks [μM]
JK07	6	M	40	2.2	1.6	43	82	444	267	150	113
SE10	15	W	12	1.1	1.1	55	47	684	65	193	108
TR21	3.5	W	13	2.5	1.6	76	88	450	659	152	95
BN15	11	M	14	1.5	1.1	66	64	656	338	54	76
HS06	9	W	11	1.4	1.4	53	68	709	635	70	48
SV14	12	W	7	0.9	1.1	32	55	1143	950	97	56
FL05	6	M	7	1.8	1.6	50	82	769	478	69	48
KS08	15	W	9	0.9	1.1	34	47	1070	976	54	61
WD24	30	M	low protein diet	0.9	0.8	21	40	942	787	93	38

from 104 ± 49 to 71 ± 27 μmol/l after 2 weeks ($p = 0.02$), (Fig. 3). There was a negative correlation between the initial mean Phe-level and the mean protein – ($p = 0.004$) and initial caloric-intake ($p = 0.08$). Significant differences between boys and girls were only found for Phe ($p = 0.02$).

Levels of the other essential amino acids were in the normal range and did not follow the levels of BCAA's.

The mean Phe-level of subgroup II ($n = 23$) was 831.4 ± 365.1 μM.

In subgroup I mean amounts of the total caloric intake before dietary modification (45 ± 14 kcal per kg body weight) were $12 \pm 5\%$ for protein, $63 \pm 8.5\%$ for carbohydrates and $26 \pm 6.5\%$ for fat. In subgroup II (unfortunately only 13 dietary records were analyzable) mean caloric intake was 61 ± 29 kcal per kg body weight, mean supply per kg body weight of protein was 1.6 ± 0.7 g of carbohydrates 9.6 ± 4.4 g of fat 1.6 ± 1 g and Phe 22 ± 10.7 mg.

The BCAA of all liver transplanted patients were in the lower range of normal (Liappis et al., 1990), the mean values and standard-deviations were for Val: 128 ± 58, Leu: 63 ± 28 and Isoleu: 33 ± 15 μmol/l (Fig. 4).

Discussion

A parameter to distinguish between dietary non-compliance and metabolic catabolism in treated PKU patients seems desirable. Ketonuria can only be observed in *overt* catabolism and thus is a weak parameter to detect *latent* catabolism in treated PKU patients. Methods and markers mentioned above to evaluate nutritional status do not seem to be practicable as routine parameters to control dietary treatment in PKU patients (Boirie et al., 2000; Chapman et al., 1997; Schulpis et al., 2000, 2004; Shenton et al., 1983).

This study was designed to detect an association between caloric intake and nutritional status on one hand and BCAA- and Phe-concentrations in plasma on the other hand in patients with classical PKU under Phe-restriction and free of infection.

That insulin lowers plasma amino acid levels has been known for many years (Luck et al., 1928). More recent studies have demonstrated a direct *in vivo* inhibition of peripheral amino acid release from resting muscle (Prozefsky et al., 1969a, b). In addition, an elevation of branched chain amino acids has been observed in diabetes and in pancreatectomized dogs (Carlsten et al., 1966; Ivy et al., 1951). Branched chain amino acids are among the amino acids most sensitive to altered systemic insulin levels (De Barnola, 1965; Zieneman et al., 1966). Thus, elevated levels

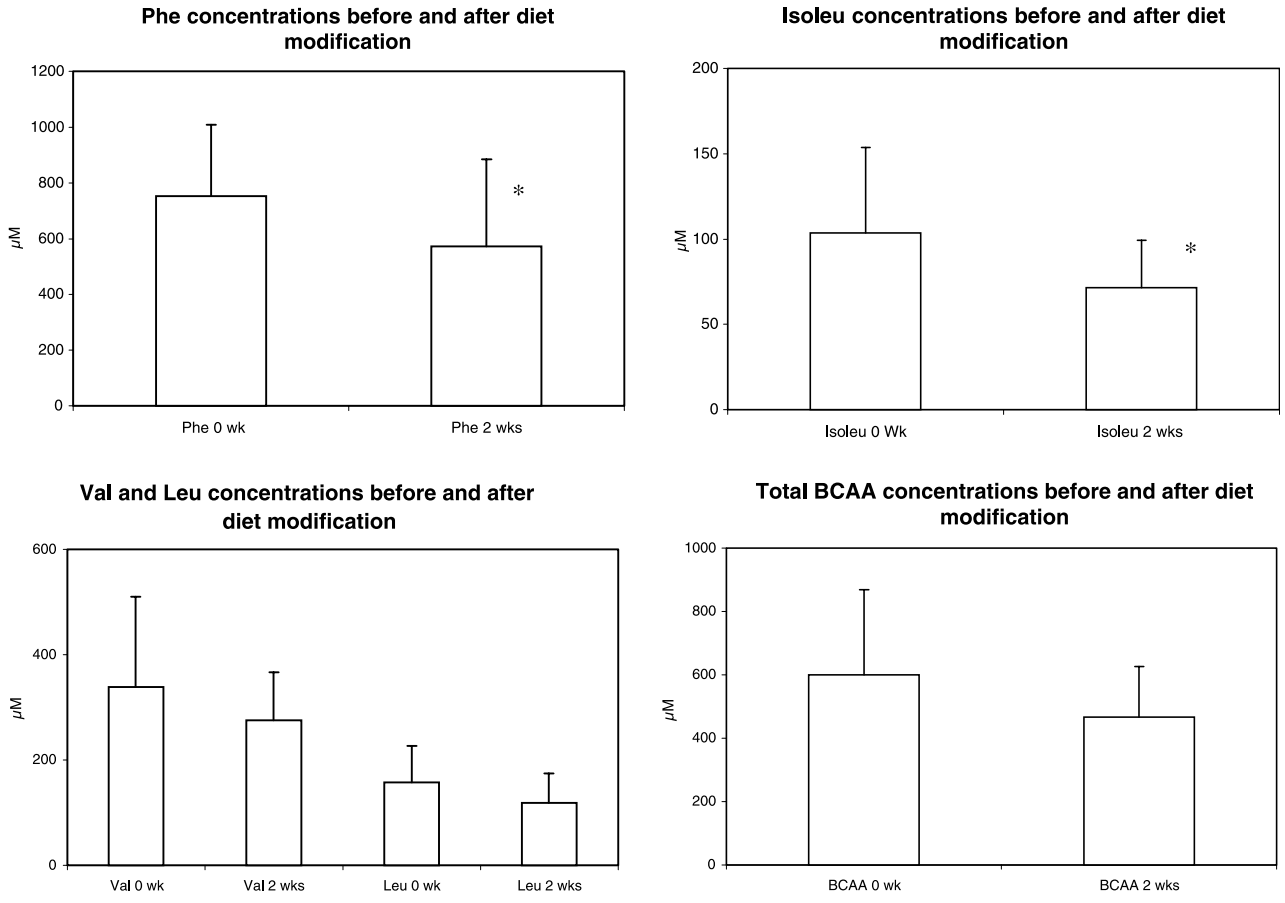

Fig. 3. Mean Phe and BCAA concentrations [μM] of subgroup I before and 2 weeks (wks) after diet modification. *Phe*, phenylalanine; *Leu, Isoleu, Val*, Leucine, Isoleucine, Valine; *BCAA*, branched chain amino acids; * = $p < 0.05$

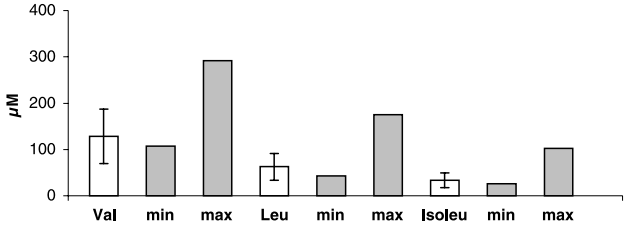

Fig. 4. Total BCAA in liver transplanted patients under high caloric and carbohydrate rich diet ±SD, and their normal range (min- and max-values)

of BCAA in early starvation may reflect a release from peripheral protein stores (Felig et al., 1969). As we could show in our liver transplanted children with high carbohydrate intake BCAA were in the lower range of normal.

In treated PKU patients the main dietary features are restriction of natural dietary protein, a protein substitute free of Phe, a sufficient vitamin and mineral supplement and a generous supply of calories. Children with PKU have the same requirements of protein and energy as children without PKU to cover their needs for growth (Przyrembel, 1996). To achieve acceptable blood Phe-concentrations an adequate caloric intake and nutritional status is important. In our study we could observe that *latent* catabolism in treated PKU patients as judged by elevated levels of BCAA is quite common and that increased caloric intake resulted in lowering of the elevated Phe- and BCAA-concentrations in most patients. BCAA levels revealed a different pattern of response, the strongest effect could be seen in the decrease of Isoleu. We found a negative correlation between the initial mean Phe-level and the mean protein- and initial caloric intake. Recent studies document patients' failure to consume an adequate amount of Phe-free protein substitute (MacDonald et al., 1997; Prince et al., 1997; Schultz and Bremer, 1995). Especially teenage or young adult patients often stop taking their protein substitute while still following a low protein diet. This could contribute to latent catabolism observed in our study.

Neither hypertyrosinaemia – which is often used as a parameter of catabolism- nor ketonuria as markers for *overt* catabolism have been observed in our study.

Energy balance could be improved by adding 'high calorie' supplements (like *Duocal*®). Alternatively, protein substitutes enriched with fat and carbohydrates (as for example *p-am ANAMIX*®) may be better suited to increase caloric intake and to avoid protein catabolism as they are more compatible with modern teenage lifestyle.

We are aware that this study has some limitations. All food and drink intake was recorded at home. Misreporting of food intake is a commonly encountered error with this method. Blood samples were drawn in the hospital's outpatient clinic or at the local pediatrician's office. They were not collected at the same standardized time of the day or related to food intake. Therefore we analyzed retrospectively blood samples of the last 6 years. We could not find a sign. difference between Phe- ($n = 172$) and BCAA- ($n = 13$) values ($p > 0.05$) collected in the hospital's outpatient clinic or at the local pediatrician's office. Spronson et al. demonstrated a rise in Phe-levels after standardized breakfast up to 145% of the initial measurement and no significant decrease in plasma Phe-levels until 2 hours after a subsequent standardized lunch. After a 12-hour overnight fast increased plasma Phe-levels up to 123% of the initial pre- breakfast level occurred. The authors concluded that the timing of blood sampling is not a significant factor for the monitoring of PKU treatment (Van Spronsen et al., 1993). Additionally, Owen et al. could show that eating a mixed meal causes only very small postprandial increases in peripheral venous plasma BCAA concentrations (Owen et al., 1969, 1980).

Conclusion

We evaluated the concentrations of BCAA as a laboratory parameter to distinguish metabolic catabolism from dietary incompliance in treated PKU patients. Latent catabolism is common in these patients. Increased caloric-intake resulted in lowering of most of the elevated Phe- and BCAA-concentrations. Elevated BCAA may help to distinguish between dietary non-compliance and metabolic catabolism.

Failure to recognize catabolism can render treatment of PKU patients difficult. For the treatment of teenage PKU patients protein supplements enriched with fat and carbohydrates seem to be promising in order to prevent muscle protein catabolism with attendant elevated plasma Phe.

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