TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2014) 44: 323-329
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doi:10.3906/sag-1302-30

Pediatric reference intervals for plasma and urine essential amino acids in a Turkish population

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Received: 09.02.2013 • **Accepted:** 01.05.2013 • **Published Online:** 15.01.2014 • **Printed:** 14.02.2014

Aim: To establish age- and sex-specific reference intervals for essential amino acids in a healthy Turkish pediatric population.

Materials and methods: A total of 945 clinically healthy children (531 boys and 414 girls, ranging in age from birth to 14 years) were enrolled. Plasma and urine amino acids' concentrations were measured by high-performance liquid chromatography.

Results: Concentrations of essential amino acids in plasma were higher in girls than in boys in the age groups of 0–1 months and 7–14 years; however, there was no difference in the other age groups. Concentrations of essential amino acids in urine were higher in girls than in boys in the age group of 0–1 months; however, there was no difference in the other age groups. Our results demonstrated the sex-related differences in concentrations of leucine, isoleucine, valine, phenylalanine, lysine, and histidine in plasma, which increased with age in boys but not in girls. The concentrations of leucine, tryptophan, methionine, and lysine in urine declined with age in girls but not in boys, which were sex-related differences, too.

Conclusion: We defined essential amino acids' reference intervals in a Turkish pediatric population.

Key words: Reference intervals, plasma, urine, essential amino acids, pediatric

1. Introduction

Amino acids are the basic structural units of proteins. The diversity of amino acids provides variability in the function and structure of proteins. Some amino acids cannot be synthesized by most mammals. Therefore, they are considered "essential" constituents of the diet for maintenance of health and growth (1). The amino acids considered essential for humans are leucine, isoleucine, valine, phenylalanine, threonine, tryptophan, methionine, lysine, and histidine (2).

Since they are not synthesized in the human body, these amino acids must be derived from the diet. Amino acids such as leucine, isoleucine, and valine are branched-chain amino acids and essential amino acids. In addition to their critical role as substrates for protein synthesis, these amino acids play a variety of roles such as energy sources and substrates in the citric acid cycle (3–6). Phenylalanine, histidine, and tryptophan are aromatic amino acids and are also essential amino acids needed for the human body. They are used for the synthesis of proteins (7).

Amino acids for their specific purposes appear in plasma and other body fluids. However, they disappear through conversion to other amino acids, breakdown, and excretion in urine. Plasma concentrations of amino acids may provide important information about metabolic processes in the body (8). Their measurement in physiological fluids provides important information for fundamental studies and the diagnosis of many pathological and inherited conditions (9–11).

For laboratory scientists and pediatricians, it is necessary to develop reference intervals for amino acids

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in plasma and urine samples, according to age and sex. The reference values of amino acids in the Turkish population have not previously been investigated in detail. To our knowledge, there are no data in pediatric reference intervals for blood and urine concentrations of essential amino acids in the Turkish population. The aim of this study was to therefore establish age- and sex-specific reference intervals for essential amino acids in a healthy Turkish pediatric population.

2. Methods

2.1. Pedriatric population

This study included plasma and urine samples collected from 945 healthy children (531 boys and 414 girls) in Ankara, Turkey, ranging in age from birth to 14 years. This project was approved by the Ethics Committee of Gülhane Military Medical Academy, Ankara, Turkey. All children were assessed for enrollment by a physician assistant at Dışkapı Children's Health and Diseases, Hematology, Oncology Training and Research Hospital in Ankara, Turkey, as part of a routine well-child examination. The children's health statuses were checked by a brief physical examination and laboratory tests, including a standard clinical chemistry profile and urinalysis. Children were excluded from the study if they had any of the following conditions: skin abnormalities, vomiting, lethargy, hypotonia, coma, seizures, facial dysmorphic features, mental retardation, known dietary or nutritional abnormalities, gastrointestinal disease, liver disease, infection, endocrine disease, cancer, fever, renal disease, or were taking medication. Children who presented with increased ammonia, pyruvate, and lactate levels, and/or if the parent did not provide consent were also excluded. Children and their families had similar socioeconomic levels. There was no racial or ethnic difference in this population. All the children's weight and height were between the 50th and 75th percentiles. Blood samples were collected following 8–10 h fasting except for those in the 0–1 months and 1–24 months age groups. In the other children, blood and spot urine samples were therefore collected without fasting. Both plasma and urine samples were stored and frozen at –80 °C until analysis.

2.2. Sample preparation

One hundred milliliters of precipitation reagent (sulfosalicylic acid) was added to 400-µL samples (both urine and plasma). All samples were vortexed for about 30 s and left for 30 min at room temperature. Then the samples were centrifuged at 10,000 rpm and at 4 °C for 5 min. The supernatants were filtered through 0.45-µm Whatman PTFE filters (Sigma-Aldrich Chemie GmbH, Munich, Germany).

2.3. Amino acid analysis

All amino acids were measured with a high pressure liquid chromatography (HPLC) system. Briefly, measurements were made in eluent units, and 6 different eluents, each with different pH values, were used (Eluent A, B, C, D, E, F, MembraPure, Bodenheim, Germany). The fluid volume of each bottle was actively monitored during the operation. The samples were stored at 8 °C in the autosampler. With a flow rate of 0.01–1.49 mL/min samples were injected with 1-µL steps by a XYZ robot into the autosampler unit. After collection in the autosampler unit, all samples were sent to a mixer passing through a cation-exchange column (Nucleosil 3 µm C18 120 Å LC Column 125 × 4 mm, Phenomenex, CA, USA). Column temperature was controlled by a Peltier cooler system and operated between 20 °C and 100 °C. During analysis, temperature was constant at 115 °C. Amino acids were separated by ion exchange chromatography and identified by reaction with ninhydrin and photometric detection by using an automatic amino acid analyzer (Aracus, MembraPure, Bodenheim, Germany). Photometers detected the separated amino acids at 570 nm and 440 nm and the signals were registered by the software aminoPeak (MembraPure GmbH, Germany). For quantification of amino acids, the peak areas used were calculated with internal standard. The internal standard was norleucine (Sigma-Aldrich Chemie GmbH, Munich, Germany). Amino acid concentrations were expressed as µmol/L for plasma and µmol/g creatinine for urine samples.

2.4. Statistics

All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) statistical package. Distributions were evaluated using the one-sample Kolmogorov–Smirnov test. Mean, standard deviation (SD), median, minimum (min) and maximum (max) values, and 2.5, 25, 50, 75, 97.5 percentiles were used for the described data. Student's t-test was used to compare the boys' and girls' plasma and urine essential amino acids' concentrations. In statistical analysis values of $P < 0.05$ were regarded as statistically significant. Reference intervals were based on the central 95% of the data, considering 95% confidence intervals (excluding the ranked values <2.5 percentile and >97.5 percentile).

3. Results

A total of 945 healthy children were enrolled in this study, including 531 boys and 414 girls, ranging in age from birth to 14 years. The recruitment of healthy children was based on the following age groups: 0–1 months; 1–24 months; 2–7 years; and 7–14 years. No sample was collected from sick children undergoing a detailed metabolic work-up. It was determined that the research project would develop complete pediatric reference intervals for essential amino acids in a Turkish pediatric population.

The recovery of the plasma and urine amino acids was between 89% and 107% as determined by mixing each

amino acid standard separately to a final concentration of 200 μmol/L in pooled plasma and urine samples, and recovery was calculated as the difference between spiked and unspiked plasma and urine samples. The intraassay coefficient of variation (CV) was between 1% and 7% as determined by replicate analysis of the pooled plasma and urine samples (n = 20) in a single run. The interassay CV was between 3% and 16% as determined by replicate analysis of the aliquots of plasma and urine samples stored at –84 °C and analyzed 10 separate times over a period of 1 month.

Mean, SD, median, min-max values, and reference intervals are shown for plasma and urine essential amino acids in Tables 1 and 2, respectively. The chromatogram of a child is shown in the Figure. Concentrations of essential amino acids in plasma were higher in girls than in boys in the age groups of 0–1 months and 7–14 years (Table 1). There was no difference in the other age groups (Table 1). However, concentrations of amino acids in urine were higher in girls than in boys in the age group of 0–1 months (Table 2). There was no difference in the other age groups (Table 2). Boys and girls exhibited their highest plasma essential amino acids' levels in the age group of 7–14 years (Table 1). On the other hand, boys and girls exhibited their highest urine essential amino acids' levels in the age group of 1–24 months (Table 2).

Our results demonstrated sex-related differences in concentrations of leucine, isoleucine, valine, phenylalanine, lysine, and histidine in plasma, which increased with age in boys, but not in girls (Table 1). Concentrations of leucine, tryptophan, methionine, and lysine in urine declined with age in girls, but not in boys. These were sex-related differences, too (Table 2). Plasma threonine showed a modest 2-step increment: the first increase occurred from birth to 1 month of age, and the second from 7 to 14 years in both sexes. Concentrations of tryptophan, methionine, and lysine in plasma showed a 1-step decrease: this occurred from 2 to 7 years in both sexes (Table 1). Concentrations of isoleucine, valine, phenylalanine, and threonine in urine showed a 1-step increment: this occurred from 1 to 24 months in both sexes (Table 2).

4. Discussion

To our knowledge, this is the first study in the Turkish children population examining plasma and urine essential amino acids. We hope that this will be a reference study for following studies about plasma and urine essential amino acids' concentrations in the Turkish pediatric population.

Plasma amino acid compositions are dependent on the intake and metabolism of essential and nonessential amino acids. Amino acid analyses are requested to diagnose the class of inborn errors of metabolism called aminoacidopathies. Increased concentrations of specific amino acids in plasma and urine samples are diagnostic (12).

There are important indications for the monitoring of blood and urine amino acids. One of them is important clinical situations such as developmental delay, intolerance to feeding, and hepatomegaly, which suggest aminoacidopathies. Another important indication is the monitoring of blood and urine amino acids to follow the effectiveness of treatment (12). Plasma and urine amino acid measurements are one of the most common tests analyzed in aminoacidopathies.

Evaluation of pediatric reference intervals is taken into account by each laboratory since the collection of samples from healthy individuals is considered a major task. Pediatric reference intervals are often difficult to establish because of the challenges related to obtaining sufficient numbers of blood and urine samples from healthy children. We studied a group of 945 healthy Turkish children, ranging in age from birth to 14 years. Our data defined reference intervals for plasma and urine essential amino acids' levels in a clinically healthy pediatric population living in Ankara, Turkey. Our results show that boys and girls had higher plasma essential amino acids' concentrations in the age group of 7–14 years. This phenomenon could be explained by increases in muscle mass according to age. Yi et al. have established a plasma amino acid reference range for younger Chinese children. Furthermore, they have reported that the 1–5 year group had higher levels of phenylalanine, valine, and isoleucine and lower levels of methionine, tryptophan, and threonine compared to the 0–1 year group (13).

Plasma amino acid concentrations are high during the first days of life, especially in premature neonates, but they tend to be low in infants with low birth weights for their gestational age because of placental insufficiency (1). Plasma amino acid concentrations vary by about 30% during the day; therefore, blood specimens should be collected at the same time each day. Values are the highest in midafternoon and the lowest in early morning. This diurnal variation is particularly important when specimens are analyzed for detection of heterozygous states of defective metabolism (1). Amino acid excretion in urine varies with maturation of renal tubular function. Premature infants, especially during the first week, have a generalized physiological renal aminoaciduria; even at full term, aminoaciduria is more pronounced compared with normal adults (1). Therefore, we collected all samples from subjects at about the same time.

Some analytes, such as plasma and urine amino acids, show an age-related distribution of their concentrations. Therefore, it is important to use appropriate reference intervals when working with a pediatric population. Thus, plasma and urine amino acid concentrations of

Table 1. Distribution of plasma essential amino acids' concentrations (μmol/L) in a Turkish pediatric population.

Age group	Sex	N		Amino acids								
				Leu	Ile	Val	Phe	Thr	Trp	Met	Lsy	His
0–1 months	Girl	27	Mean ± SD	102.5 ± 29.5	52.3 ± 15.6	147.4 ± 29.0	58.4 ± 22.1	107.3 ± 31.9	47.0 ± 14.0	21.5 ± 9.4	140.3 ± 41.8	70.8 ± 25.7
			Median (Min-Max)	103.0 (47.4–158.0)	52.6 (24.9–86.4)	146.7 (81.3–195.4)	57.2 (31.8–105.5)	103.8 (56.4–194.6)	49.8 (23.4–68.1)	19.2 (10.6–47.8)	144.8 (65.8–227.7)	74.9 (9.4–110.6)
			Ref. range	47.4–158.0	24.9–86.4	81.3–195.4	31.8–105.5	56.4–194.6	23.4–68.1	10.6–47.8	65.8–227.7	9.4–110.6
	Boy	34	Mean ± SD	88.5 ± 4.4	50.6 ±14.4	120.7 ± 38.1	55.3 ± 21.1	107.6 ± 35.9	40 ± 12.1	23.1 ± 11.1	126 ± 42.9	66.8 ± 24.1
			Median (Min-Max)	87.21 (50.27–140.4)	51.5 (23.4–84.8)	111.9 (64.91–195)	48.51 (21.72–106.8)	103.35 (57.45–185.69)	39.905 (20.17–67.41)	19.35 (10.97–50.11)	120.1 (53.39–222.9)	67.83 (15–118.7)
			Ref. range	50.3–104.4	23.4–84.8	64.9–195.0	21.7–106.8	57.5–185.6	20.2–67.4	11.0–50.1	53.4–222.9	15.0–118.7
			P	0.064	0.684	0.010	0.706	0.933	0.058	0.635	0.245	0.396
1–24 months	Girl	49	Mean ± SD	98.4 ± 24.1	57.9 ± 16.1	162.8 ± 50.5	61.6 ± 21.4	102.0 ± 32.1	47.1 ± 12.0	22.5 ± 9.4	139.6 ± 33.9	78.1 ± 18.1
			Median (Min-Max)	95.9 (56.2–146.3)	56.2 (29.06–91.4)	155.3 (79.25–264.6)	60.0 (26.8–119.5)	103.2 (38.7–159.9)	47.5 (23.9–68.7)	20.3 (9.0–43.7)	135.8 (59.4–199.4)	80.1 (33.6–109.9)
			Ref. range	60.4–145.0	31.8–88.2	81.8–260.3	29.4–106.5	42.0–154.8	25.3–68.4	9.3–41.7	70.3–195.7	38.4–107.6
	Boy	41	Mean ± SD	99.0 ± 25.1	57.1 ± 15.9	160.0 ± 46.9	59.7 ± 20.8	95.5 ± 32.2	45.0 ± 12.3	21.8 ± 9.3	130.2 ± 35.3	77.2 ± 17.5
			Median (Min-Max)	97.2 (55.6–148.8)	54.0 (29.6–91.8)	155.9 (79.1–266.6)	56.9 (26.3–116.5)	93.1 (35.1–159.8)	43.1 (23.2–69.0)	19.0 (9.3–43.8)	128.3 (57.5–197.4)	78.3 (32.5–109.8)
			Ref. range	58.8–143.9	31.0–88.2	84.5–263.9	27.5–104.7	39.9–155.9	23.6–68.3	9.7–42.7	61.2–194.7	45.1–106.2
			P	0.860	0.590	0.660	0.336	0.040	0.096	0.449	0.008	0.586
2–7 years	Girl	120	Mean ± SD	106.0 ± 29.6	56.9 ± 13.5	176.4 ± 49.9	60.7 ± 26.9	92.0 ± 23.6	38.1 ± 18.0	18.5 ± 5.2	132.8 ± 31.0	78.7 ± 17.2
			Median (Min-Max)	107.1 (55.7–163.9)	56.1 (32.5–82.2)	170.8 (91.2–275.3)	58.0 (16.3–145.5)	90.4 (50.2–137.8)	36.4 (5.2–69.8)	16.8 (11.2–28.8)	128.3 (77.3–199.9)	79.4 (42.0–105.3)
			Ref. range	57.3–163.0	32.6–81.5	97.9–273.4	20.0–138.4	52.1–133.3	5.9–68.5	11.2–28.8	83.8–197.7	42.5–104.0
	Boy	163	Mean ± SD	104.6 ± 29.4	55.9 ± 13.4	179.2 ± 50.2	64.5 ± 23.2	91.3 ± 24.1	41.4 ± 15.3	19.5 ± 5.1	133.3 ± 31.6	82.0 ± 16.3
			Median (Min-Max)	99.4 (55.4–163.0)	55.3 (31.7–83.0)	173.7 (96.9–277.5)	57.5 (23.2–141.6)	89.1 (50.5–135.3)	41.3 (5.3–68.3)	19.9 (11.1–29.9)	131.6 (79.0–198.7)	84.4 (42.5–105.4)
			Ref. range	58.5–162.0	33.8–80.6	99.8–272.4	32.4–117.6	51.9–133.4	9.7–67.7	11.3–29.8	79.8–198.1	44.9–105.2
			P	0.698	0.617	0.734	0.154	0.796	0.198	0.331	0.901	0.318
7–14 years	Girl	293	Mean ± SD	105.1 ± 29.0	56.8 ± 17.1	179.5 ± 45.1	64.8 ± 25.6	121.5 ± 31.4	41.0 ± 13.5	21.6 ± 6.7	139.8 ± 32.6	85.5 ± 15.9
			Median (Min-Max)	103.6 (55.7–161.0)	53.9 (31.2–92.6)	172.6 (84.9–272.1)	58.0 (38.1–163.8)	117.0 (73.0–191.8)	40.6 (7.6–67.0)	20.1 (11.1–35.3)	138.9 (88.5–217.4)	92.4 (51.4–104.3)
			Ref. range	56.7–160.1	31.9–92.3	92.3–271.1	38.2–152.3	73.3–189.9	8.6–66.1	11.1–35.3	89.0–211.2	51.4–104.3
	Boy	161	Mean ± SD	111.4 ± 27.0	59.4 ± 14.2	191.2 ± 42.3	66.1 ± 18.5	124.2 ± 30.1	43.4 ± 16.1	22.9 ± 6.0	146.4 ± 36.8	81.4 ± 15.8
			Median (Min-Max)	110.5 (58.9–167.7)	59.6 (31.2ü90.8)	192.5 (104.4–274.6)	64.4 (38.3–124.7)	123.9 (73.3–183.4)	44.7 (5.6–69.1)	21.4 (12.5–35.2)	148.4 (82.7–218.7)	84.5 (49.7–104.3)
			Ref. range	61.8–165.5	32.2–90.2	108.3–273.7	39.0–117.7	73.7–182.5	5.6–66.7	12.5–34.9	86.1–216.6	49.8–104.3
			P	0.200	0.246	0.160	0.163	0.589	0.219	0.272	0.314	0.263

Leu: leucine; Ile: isoleucine; Val: valine; Phe: phenylalanine; Thr: threonine; Trp: tryptophan; Met: methionine; Lsy: lysine; His: histidine.

Table 2. Distribution of urine essential amino acids' concentrations (μmol/g creatinine) in a Turkish perdiatric population.

Age group	Sex	N		Amino acids								
				Leu	Ile	Val	Phe	Thr	Trp	Met	Lsy	His
0–1 month	Girl	27	Mean ± SD	82.9 ± 65.3	56.8 ± 45.9	59.3 ± 57.4	80.4 ± 61.0	246.4 ± 215.6	114.8 ± 166.6	69.6 ± 52.0	160.6 ± 74.8	1.194.5 ± 873.7
			Median (Min-Max)	61.1 (10.2–176.3)	47.2 (7.6–143.2)	40.0 (7.0–188.2)	65.3 (3.5–187.9)	244.2 (22.8–721.0)	85.8 (10.7–641.1)	59.6 (2.8–186.2)	124.8 (68.1–266.5)	937.9 (168.7–3.026.0)
			Ref. range	10.2–176.3	7.6–143.2	7.0–188.2	3.5–187.9	22.8–721.0	10.7–641.1	2.8–186.2	68.1–266.5	168.7–3.026.0
	Boy	34	Mean ± SD	40.3 ± 41.0	48.2 ± 42.6	38.9 ± 77.0	36.5 ± 27.3	234.7 ± 238.9	40.0 ± 39.0	57.3 ± 36.4	108.7 ± 88.0	601.4 ± 471.1
			Median (Min-Max)	28.1 (4.0–151.2)	37.6 (3.8–124.4)	17.6 (1.7–292.3)	32.4 (5.3–75.6)	161.6 (5.1–650.0)	24.3 (4.0–140.8)	57.1 (4.6–120.9)	92.1 (11.7–236.6)	671.0 (42.3–1766.0)
			Ref. range	4.0–151.2	3.8–124.4	1.7–292.3	5.3–75.6	5.1–650.0	4.0–140.8	4.6–120.9	11.7–236.6	42.3–1.766.0
			P	0.058	0.593	0.096	0.052	0.497	0.099	0.590	0.160	0.048
1–24 months	Girl	49	Mean ± SD	73.7 ± 56.3	68.3 ± 47.8	79.5 ± 68.5	130.2 ± 90.4	264.8 ± 213.3	95.7 ± 72.7	55.9 ± 39.2	130.9 ± 59.0	1.500.4 ± 940.0
			Median (Min-Max)	56.9 (1.2–308.4)	58.8 (1.7–252.0)	65.7 (1.4–435.5)	116.2 (3.9–430.09	223.0 (3.7–1096.0)	76.3 (2.5–270.6)	44.7 (0.6–174.2)	138.5 (4.3–219.8)	1.330.0 (20.1–3818.0)
			Ref. range	4.2–229.4	7.5–188.4	4–256.4	6.5–373.6	7.9–886	4.3–259	5.1–149.3	6.2–216.5	88.6–3544.6
	Boy	41	Mean ± SD	74.6 ± 51.5	66.2 ± 48.1	79.6 ± 69.9	137.9 ± 94.5	250.2 ± 186.8	98.5 ± 66.9	66.6 ± 48.8	134.4 ± 55.0	1.542.6 ± 822.8
			Median (Min-Max)	67.4 (3.5–294.3)	54.5 (4.6–243.6)	60.0 (3.2–434.8)	117.2 (3.6–430.6)	209.4 (3.2–1105.0)	81.9 (1.5–273.1)	54.5 (3.1–215.4)	143.4 (0.5–218.8)	1.426.5 (39.8–3800.0)
			Ref. range	6.8–207	8.1–210.61	8–304.3	9–382.8	15.3–739.605	9.2–256.0	7–197.7	12.9–216.6	192.3–3316.5
			P	0.531	0.571	0.937	0.455	0.797	0.466	0.067	0.688	0.342
2–7 years	Girl	120	Mean ± SD	52.3 ± 44.1	44.9 ± 34.4	54.2 ± 42.3	103.5 ± 68.4	167.4 ± 120.5	86.6 ± 54.2	46.4 ± 39.1	115.8 ± 42.8	1.256.6 ± 906.1
			Median (Min-Max)	42.4 (4.6–202.3)	37.1 (4.9–161.5)	44.4 (3.2–227.1)	87.4 (11.5–321.5)	129.0 (9.3–547.3)	73.8 (8.3–219.8)	34.3 (2.2–186.3)	113.4 (12.2–193.5)	955.8 (244.7–3815.0)
			Ref. range	5–195.6	6.5–156.1	4.8–207.9	15.4–316.2	10.7–478.1	8.7–213.8	3.4–161.3	15–193.3	303.3–3758.1
	Boy	163	Mean ± SD	51.6 ± 31.5	39.5 ± 25.8	66.9 ± 56.2	124.5 ± 72.1	176.0 ± 125.0	93.1 ± 55.0	46.9 ± 33.1	126.0 ± 51.1	1.309.2 ± 741.6
			Median (Min-Max)	50.8 (5.1–143.1)	33.7 (3.0–139.0)	48.8 (1.8–313.6)	107.8 (10.2–313.6)	155.1 (15.2–810.0)	88.9 (1.1–219.5)	40.8 (3.8–163.4)	136.9 (9.8–196.0)	1.201.5 (88.5–3787.0)
			Ref. Range	8–134.3	5.6–98.8	9.9–248.2	15.4–300.1	22.2–504.8	20.3–211.8	4.8–131.2	14.9–195.9	237.8–3373.4
			P	0.358	0.587	0.148	0.028	0.474	0.460	0.532	0.191	0.163
7–14 years	Girl	293	Mean ± SD	45.4 ± 32.4	36.3 ± 25.7	56.5 ± 53.5	87.2 ± 50.8	160.1 ± 118.0	77.5 ± 52.9	33.5 ± 23.7	117.5 ± 49.9	897.7 ± 484.7
			Median (Min-Max)	32.6 (3.9–140.0)	27.8 (4.1–106.4)	38.0 (2.3–258.1)	72.2 (8.8–216.0)	120.3 (19.1–650.2)	62.6 (4.9–213.7)	27.3 (3.9–105.6)	108.1 (18.8–208.5)	858.2 (54.6–1979.0)
			Ref. Range	4.7–125.7	5.8–102.6	3.3–216.2	14.2–204.9	20.6–466.2	10–212.9	4.7–102.4	20.6–204.9	228.3–1962
	Boy	161	Mean ± SD	42.5 ± 26.1	34.7 ± 22.0	49.5 ± 40.5	84.6 ± 46.9	155.0 ± 100.2	74.9 ± 47.3	28.1 ± 18.7	124.2 ± 46.5	926.5 ± 418.8
			Median (Min-Max)	35.5 (6.6–151.3)	30.1 (1.9–104.8)	36.6 (1.8–217.8)	77.0 (5.4–214.9)	133.4 (9.8–760.1)	66.4 (5.4–185.9)	26.1 (1.7–106.2)	121.2 (24.2–208.1)	867.3 (134.3–1979.0)
			Ref. range	7.4–111.3	8.1–90.1	5.2–162.6	11.4–201.3	24.4–423	11.6–181.5	3.4–81	26–207.2	204.8–1899.4
			P	0.973	0.845	0.702	0.952	0.607	0.909	0.200	0.360	0.523

Leu: leucine; Ile: isoleucine; Val: valine; Phe: phenylalanine; Thr: threonine; Trp: tryptophan; Met: methionine; Lsy: lysine; His: histidine.

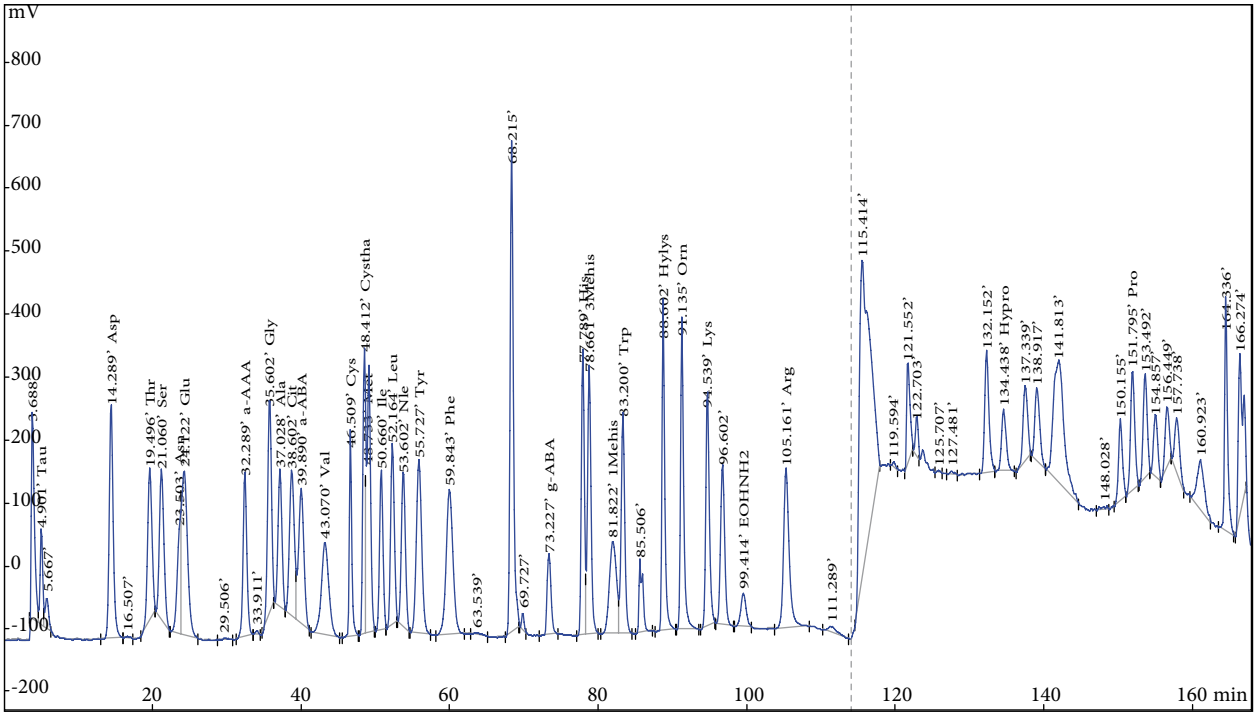

Figure. Sample chromatogram of a healthy child.

patients with a particular metabolic disease, especially aminoacidopathies, can be easily compared from infancy to adolescence with those of the healthy control population of the appropriate age.

There were some limitations of this study. Firstly, the data were usually divided into various age groups, a primordial step that contributed to a decrease in the number of studied controls per defined category. The numbers of cases in the 0–1 month and 1–24 month groups were smaller than those in the other age groups. Besides that, the power of comparisons was not low. According to our power analysis, the lowest power value is 74.5% for leucine comparison. Secondly, the dietary status of the healthy controls was unknown. Some amino acids may be affected by the duration of fasting and/or dietary regimens such as breastfeeding and formula feeding.

In conclusion, we were able to establish pediatric essential amino acids' reference intervals in subjects from birth through 14 years with HPLC by ninhydrin postcolumn derivatization. Significant differences were observed among ages and sexes. The reference intervals derived in the present study may be useful when applied to the diagnosis and monitoring of therapy in patients with a particular metabolic disease in pediatric subjects.

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